Understanding and planning for the potential impacts of OsHV-1 µVar on the Australian Pacific oyster industry (FRDC 2011/043)

Final Report

1 September 2012







Australian Government

Fisheries Research and Development Corporation



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Final Report

Tom Lewis, Don Defenderfer, Bruce Zippel

Project No: FRDC 2011/043

September 2012



Australian Government

Fisheries Research and Development Corporation





Title: Understanding and planning for the potential impacts of OsHV-1 μ Var

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FRDC Project No: 2011/043

Date: September 2012

Published by: RDS Partners Pty Ltd, 2012.

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ISBN: 978-0-9872175-2-3

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1 Non-Technical Summary

2011/043: Aquatic Animal Health Subprogram: understanding and planning for the potential impacts of OsHV-1 μ Var on the Australian Pacific oyster industry

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OBJECTIVES

- 1. A desktop study on industry relevant issues associated with the OsHV-1 μvar virus and the related Pacific Oyster Mortality Syndrome (POMS)
- 2. A field visit to France by a small group of industry representatives to discuss firsthand the French industry and regulatory experience regarding the effects and management of OsHV-1 μ Var.
- 3. The development and extension of a national strategy to control and/or minimise the spread of OsHV-1 μ Var in Australia and to develop management strategies to mitigate the effects of the disease in areas in which is, or may become, established.

OUTCOMES ACHIEVED TO DATE

The project outputs have contributed to or will lead to the following outcomes:

- 1. Improved knowledge within the Australian Pacific oyster and related sectors regarding the source, transmission, effects, mitigation and control of OsHV-1 μ Var which will inform consideration of the best return on investment on management and control measures (e.g. selective breeding, tightened biosecurity, management strategies).
- 2. Improved collaboration between Australian industry, scientists and regulators regarding management of OsHV-1 µVar in Australia.
- 3. Improved industry and regulator readiness to combat new outbreaks should they occur in Australian waters.

NON-TECHNICAL SUMMARY

This report summarises current understandings and describes desired outcomes and actions regarding four key issue areas associated with the Pacific Oyster Mortality Syndrome (POMS), the name that has been given to the OsHV-1 μ Var - mediated viral disease associated with high mortality events in the Pacific oyster (*Crassostrea gigas*).

This project was initiated through discussions between FRDC and the Shellfish Industry

Council of Australia in response to the threat posed to the Australian Pacific oyster industry by the incursion of the POMS virus - which has been responsible since at least 2008 for devastating mortalities of 80-100% of farmed oysters in parts of Europe, France and New Zealand.

In 2010 the virus was identified in New Zealand where it has had major impacts upon the oyster industry; in late 2010 the disease was found in dead and dying Pacific oysters in two estuaries in NSW. The virus is now listed as notifiable disease.

In 2011 Australian industry, scientists and regulators recognised the massive threat that this virus posed to the Australian Pacific oyster farming industry. The urgent need for a national strategy to understand, control and/or mitigate the effects of this virus was prompted by industry, scientists and regulators; a National POMS Advisory Group was formed to guide a strategic way forward.

FRDC funding was received in July 2011 for a project to help industry understand the disease so as to be able to mitigate and prevent its spread to other oyster growing regions in Australia.

The project's first stage, a desktop analysis and subsequent development of extension materials (see Q & A Fact Sheets in Current Position and Future Plans for the Australian Industry report within Appendix 3 and <u>on line</u>) was significantly informed by the deliberations and Final Report of the International OsHV-1 μ Var Workshop that followed the Aquatic Animal Health Conference in Cairns in July 2011 (see within Appendix 3 and <u>on line</u>) and was released in November 2011.

The second stage of the project, the study tour to France, significantly increased the understanding of the oyster industry about the adverse affects of POMS and the strategies, including extension, research and development that are needed if we hope to successfully combat the virus in Australia. The report of the France study tour is available <u>on line</u> as well as within Appendix 3 and significantly informs this strategy.

Following the study tour, the project's third objective was amended slightly to focus more on pragmatic industry needs.

The third stage of this project, the development of a POMS strategy document (see Current Position and Future Plans for the Australian Industry report within Appendix 3), focuses on: the potential movement of the virus into other Pacific oyster production areas around Australia and the need to summarise current information and desired outcomes and actions; a discussion of current understandings and opinions regarding POMS by industry and government; and making recommendations regarding four key industry issues associated with the virus, including

- Emergency Response Protocols
- Tracking Oyster Movements
- Monitoring POMS
- Hatchery Protocols

There has been extensive consultation with growers from all states, DAFF and AAHL during the development of this project and the identification of the four key issues above.

The Current Position and Future Plans for the Australian Industry report is based on research, interviews with key industry and government stakeholders and input from the Oysters Australia National POMS advisory group.

Recommendations are detailed in the Current Position and Future Plans for the Australian Industry report (Appendix 3).

It is recognized that the final strategy document's recommendations will need to be accepted and endorsed by appropriate industry, science and regulatory agencies before the next phase of POMS investment, extension and research can commence. It is also recognized that a considerable amount of research and progress towards strategically *managing* POMS has been undertaken (and is the process of being undertaken) since the original project application was developed in 2011.

Key words: Pacific Oyster Mortality Syndrome, POMS, OsHV-1 μ Var, Current Position and Future Plans, French Study Tour Report, Q & A Fact Sheets.

2 Acknowledgements

This report was funded by the Fisheries Research and Development Corporation (FRDC) as part of project number 2011/043 (*Aquatic Animal Health Subprogram: understanding and planning for the potential impacts of* OsHV-1 μ Var *on the Australian Pacific oyster industry*).

The FRDC is Australia's leading agency concerned with planning, investing in, and managing fisheries research, development and extension.

The FRDC is a statutory corporation founded in 1991 under the Primary Industries and Energy Research and Development (PIERD) Act 1989. It is responsible to the Minister for Agriculture Fisheries and Forestry.

The FRDC's mission is to maximise economic, environmental and social benefits for its stakeholders through effective investment and partnership in research, development and extension.

The project was managed by Tom Lewis and Don Defenderfer at RDS Partners. The Current Position and Future Plans for the Australian Industry report was overseen and given key input by members of the National POMS Advisory Group. Morag Anderson assisted in the editing of the final report.

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3 Background

This project was initiated through discussions between the FRDC and the Shellfish Industry Council of Australia in response to the serious threat posed to the Australian Pacific oyster industry by the incursion of the OsHV-1 μ Var virus - which has been responsible for mortalities of 80-100% of farmed oysters in countries in which it is established.

The virus has been in Europe for some years, and has had a dramatic adverse effect on the French Pacific oyster farming industry. In 2010 the virus was identified in dead and dying oysters in New Zealand and in late 2010 and early 2011 OsHV-1 μ Var was found in dead and dying Pacific oysters in NSW.

	FRANCE	UK AND EUROPE	AUSTRALIA	NEW ZEALAND	REST OF THE WORLD
First outbreak	April 2008	UK: July 2009 IRE: 2009 NL: June 2011	Nov 2010	March 2010	None reported
Growing areas affected	100%	66% Ireland 3% England	20% in NSW* 1% nationally by lease area	73%	
Spat mortality <12mths	High	High	Highest	80–100%	
Juvenile mortality 12–18mths	Medium	Medium	Higher	25-42%	
Adult mortality >18 mths	Low	Low	High	8–20%	
Is there a decrease in the impact of disease over time	No (same)	No (same)	Not applicable	Exposed populations less affected than naïve	
Economic Impact to date	Not apparent but little available data		10% estimated loss for NSW oyster production	Farm production fell by 25%	
Potential future impact			SA and Tas: Very high due to SA reliance on one hatchery in Tasmania and plan to expand export markets	Current 30% export to AUS, 30% to SEA, 30% to Pacific. Listing of disease may affect exports	

Summary of impact in different locations:

From: Final Report OsHV-1 µVar International Workshop, 2011 (see report within Appendix 3)

FRDC funding was received in July 2011 for this project to help industry understand the disease so as to be able to mitigate and prevent its spread to other oyster growing regions in Australia.

4 Need

The Pacific oyster virus (Ostreid herpesvirus-1, OsHV-1 μ Var) is a pathogen that has been regularly detected in France since 1991. The virus has generally been associated with Pacific oyster larval mortality in hatcheries and in Pacific oyster spat mortality outbreaks.

The 2010 incursion of the highly pathogenic OsHV-1 μ Var micro variant into New Zealand and NSW waters, leading to 80-100% mortality in weeks, raised the very real prospect of this deadly oyster virus spreading to other Pacific oyster growing states.

This virus, if spread unchecked in Australia, has the potential to destroy the Pacific oyster aquaculture industry, which is currently worth about \$65 million in farm gate sales in SA, Tasmania and NSW.

It was recognized by key stakeholders that there was an urgent need to collate and disseminate information regarding the source, transmission, pathogenicity, control and mitigation of this virus and its effects on farmed Pacific oysters.

The urgent need for a national strategy to understand, control and/or mitigate the effects of this virus was what prompted industry, scientists and regulators to seek funding support from the FRDC for this project.

A need for a nationally coordinated response was recognized and a national POMS Advisory Group was formed to guide a strategic way forward regarding the virus.

The project directly addresses the FRDC Strategic theme *Biosecurity and aquatic animal health* in that it seeks to protect the Australian Pacific oyster industry from a lethal exotic virus.

Australian industry, scientists and regulators continue to recognize the massive threat that the virus poses to the Australian Pacific oyster farming industry.

The threat of the virus spreading further in NSW and also into Tasmania and South Australia remains as does the need for an integrated and planned approach.

5 Objectives

The project was established with three main objectives:

- 1. A desktop study on industry relevant issues associated with the OsHV-1 μ Var virus and the related Pacific Oyster Mortality Syndrome (POMS).
- 2. A field visit to France by a small group of industry representatives to discuss firsthand the French industry and regulatory experience regarding the effects and management of OsHV-1 μ Var.
- 3. The development and extension of a national strategy to control and/or minimise the spread of OsHV-1 μ Var in Australia and to develop management strategies to mitigate the effects of the disease in areas in which is, or may become, established.

6 Methods

This project was developed through a variety of social research methods including workshops, field consultations, 1:1 interviews, desktop research and research (via phone and email) with key industry and government stakeholders including input from the Oysters Australia POMS Advisory Group.

The OsHV-1 μ Var International Workshop final report (included within Appendix 3) was developed through workshop input, comprehensive consultation and desktop research.

The French Study Tour Report was developed after a research trip to France that included meetings with industry and government representatives and visits to oyster growing areas and facilities. Web page blogs were also used as an interactive way to generate data and extend the findings of the French tour research.

The extension strategy and resulting Q & A Fact Sheets (included in Appendix 3) were developed through desktop research, phone and email consultation/input.

The final strategic document (Current Position and Future Plans for the Australian Industry) was developed through desktop research, phone and email consultation with industry and government.

7 Results/Discussion

This report summarises current understandings and *opinions* and describes desired outcomes and actions regarding four key issue areas associated with the Pacific Oyster Mortality Syndrome (POMS), the name that has been given to the OsHV-1 μ Var mediated viral disease associated with high mortality events in the Pacific oyster (*Crassostrea gigas*). (The term POMS relates to mortalities of Pacific Oysters under varying environmental conditions and when infected by the virus. For the sake of this document, the use of the word POMS can also mean the presence of the virus.)

Four key emergency animal disease response issue areas frame this report:

- 1. Emergency Response Protocols
- 2. Tracking Oyster Movements
- 3. Monitoring POMS
- 4. Hatchery Protocols

This report also summarizes the recommendations made by the French Study Tour component of this project (see Appendix 3) and gives an update on those recommendations, particularly on the research needs identified by the study group.

The document is based on research, interviews with key industry and government stakeholders and input from the Oysters Australia POMS advisory group.

Recommendations for action and strategic planning include:

7.1 Emergency Response Protocols

Key recommendations regarding emergence response protocols include:

- 1. Need to finalize membership and roles of national industry/government Emergency Response Group.
- 2. Government and industry to work together on emergency response scenarios and discuss on-going surveillance processes and Emergency Response Plans.
- 3. Increase communication between government and industry regarding Emergency Response protocols and requirements (eg clarification of % of mortalities that requires reporting in each state.)
- 4. Increase communication with New Zealand industry and government representatives regarding their emergency disease response processes and rebuilding strategies.
- 5. Develop a plan (or plans) for preparing OsHV-1 μ Var -focussed, industry-endorsed emergency response plan/s in SA, NSW and TAS. This plan (or plans) should include details of agreed:
 - a) technical response options, including contingency planning and learning how to live with the disease and knowing what the options are for maintaining commercial production in an infected area;
 - b) regulatory response options;
 - c) financial options available at the individual farm level and at an industry level, including sources of assistance during the recovery phase; and

d) social and other sources of support for producers coping with this sort of problem.

7.2 Tracking Oyster Movements

Major recommendations regarding ways to better understand and improve the tracking of Oyster movements include:

- 6. Complete a Gap Analysis of state tracking systems and make recommendations for improvements.
- 7. Undertake a Gap Analysis and assessment of the need for state systems to be linked to a national tracking system.
- 8. Undertake an immediate risk assessment on likely vectors of transferring the virus within a state and between states.

7.3 Monitoring POMS

Recommendations to improve communication and achieve an on-going and adequate oyster mortality monitoring system in each state include:

- 9. Recommendations developed jointly by industry and government regarding appropriate level of *active* monitoring and surveillance for POMS.
- 10. Industry and Government communication and extension strategies developed to address key issues (eg to ensure growers know correct % of mortalities that requires reporting).
- 11. Industry and government to work together to encourage industry to report mortality events when appropriate.
- 12. Investigate the use of sentinel populations in high risk areas of potential viral infection. This may involve a mixture of cultivated and feral oyster populations.
- 13. Develop national capacity and capability to report and monitor non-harvest stock movements between states and within each state.

7.4 Hatchery Protocols

Major recommendations regarding hatcheries and hatchery protocols include:

- 14. Undertake a Gap analysis regarding the need for national testing protocols for hatcheries.
- 15. Investigate the development of national hatchery protocols regarding testing for POMS if demonstrated by Gap analysis.
- 16. Increase strategic extension to industry regarding emergency response scenarios and monitoring protocols for hatcheries.

The complete results and discussions regarding this project are included in **Appendix 3** as a standalone report (Current Position and Future Plans for the Australian Industry) which will be distributed widely to industry and stakeholders.

The report in Appendix 3 also includes as attachments the Cairns 2011 Final Report OsHV-1 μ Var International Workshop (which serves to fulfil the original desktop study planned for this project), POMS Q & A Fact Sheets (which have been distributed to industry and government) and the complete French Study Report (which was also distributed widely).

8 Benefits

The benefits identified in the original application include:

- Improved knowledge within the Australian Pacific oyster and related sectors regarding the source, transmission, effects, mitigation and control of OsHV-1 μ Var which will inform consideration of the best return on investment on management and control measures (e.g. selective breeding, tightened biosecurity, management strategies).
- Improved collaboration between Australian industry, scientists and regulators regarding management of OsHV-1 μ Var in Australia.
- Improved industry and regulator readiness to combat new outbreaks should they occur in Australian waters.

All of the above benefits have been realized and will be further extended by the publication of this report and the distribution of the Current Position and Future Plans for the Australian Industry report (Appendix 3).

The Cairns 2011 OsHV-1 μ Var International Workshop report significantly raised the profile of OsHV-1 μ Var in Australia as well as providing an accessible baseline documentation of the state of knowledge about the virus. The report was an important stimulus to improved collaboration between industry, scientists and regulators regarding management of OsHV-1 μ Var in Australia.

The Q & A Fact Sheets which were developed (both short and long versions) and were distributed widely throughout industry and government not only improved the knowledge of stakeholders, but improved the readiness of the industry and regulators to combat OsHV-1 μ Var.

The French Study Report also significantly improved the knowledge of stakeholders as well as increasing national and international collaboration regarding OsHV-1 μ Var management and research issues.

9 Further Development

Activities and other steps that will be undertaken to further develop or disseminate the results of the research include:

- Placement of final report on industry web sites (eg Oysters Australia, Oysters Tasmania, SA Oysters Growers Association, NSW Farmers)
- Email notification to industry and government networks of final report publication and availability.
- Media release following publication of final report.
- Presentation of project results and recommendation at industry conferences.
- Carriage of the recommendations made in the Current Position and Future Plans for the Australian Industry report through the Oysters Australia National POMs Advisory Group.

Original data generated from the project will be stored by <u>RDS Partners</u> in electronic and hard copy versions.

10 Planned Outcomes

The project's outputs (products produced) have contributed to the planned outcomes as follows:

- A desktop study on industry relevant issues associated with OsHV-1 μVar and the related Pacific Oyster Mortality Syndrome (POMS). The *Final Report OsHV-*1 μVar International Workshop serves to satisfy this output. The outcome of this report has been: improved knowledge within the Australian Pacific oyster and related sectors regarding the source, transmission, effects, mitigation and control of OsHV-1 μVar which will inform consideration of the best return on investment on management and control measures (e.g. selective breeding, tightened biosecurity, management strategies).
- French Study Tour Report: Improved knowledge of stakeholders as well as improved collaboration between Australian industry, scientists and regulators regarding management of OsHV-1 µVar in Australia.
- **Q & A Fact Sheets (short and long versions):** Improved knowledge and awareness as well as improved industry and regulator readiness to combat new outbreaks should they occur in Australian waters.
- **Current Position and Future Plans for the Australian Industry report:** Improved collaboration, knowledge and awareness by industry, researchers and government as well as improved industry and regulator readiness to combat new outbreaks should they occur in Australian waters.

11 Conclusion

This project was initiated with a sense of urgency by industry (and supported by government) as has been articulated that the Pacific Oyster Mortality Syndrome potentially represents the biggest individual threat to Australian oyster production that it has ever faced.

In a considered approach the project at the outset identified a number of priority needs it sought to address, including:

- The urgent need for a national strategy to understand, control and/or mitigate the effects of the virus.
- An urgent need to collate and disseminate information regarding the source, transmission, pathogenicity, control and mitigation of this virus and its effects on farmed Pacific oysters.

Specific objectives were established to meet these needs:

- A desktop study on industry relevant issues associated with OsHV-1 μ Var and the related Pacific Oyster Mortality Syndrome (POMS).
- A field visit by a small group to France to engage their industry and to discuss firsthand industry experience regarding the effects and management of OsHV-1 μ Var.
- The development and extension of a national strategy to control and/or minimise the spread of OsHV-1 μ Var in Australia and to develop management strategies to mitigate the effects of the disease in areas in which it is (or may become) established.

The project achieved these objectives through the production of three key outputs (<u>French</u> <u>Study Tour Report</u>, <u>Q & A Fact Sheets</u> and the Current Position and Future Plans for the Australian Industry report) – which are included in Appendix 3.

The outcomes from the production of these three documents includes improved knowledge of stakeholders, improved collaboration between industry, scientists and regulators regarding the management of OsHV-1 μ Var in Australia, and improved industry and regulator readiness to combat new outbreaks should they occur in Australian waters.

Significantly, the project has identified research priorities, described progress made against these priorities and made recommendations for future actions regarding a strategic way forward to control and/or mitigate the effects of the virus.

Recommendations for action and strategic planning include:

11.1 Emergency Response Protocols

Key recommendations regarding emergence response protocols include:

1. Need to finalize membership and roles of national industry/government Emergency Response Group.

- 2. Government and industry to work together on emergency response scenarios and discuss on-going surveillance processes and Emergency Response Plans.
- 3. Increase communication between government and industry regarding Emergency Response protocols and requirements (eg clarification of % of mortalities that requires reporting in each state.)
- 4. Increase communication with New Zealand industry and government representatives regarding their emergency disease response processes and rebuilding strategies.
- 5. Develop a plan (or plans) for preparing OsHV-1 μ Var -focussed, industry-endorsed emergency response plan/s in SA, NSW and TAS. This plan (or plans) should include details of agreed:
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 - b) regulatory response options;
 - c) financial options available at the individual farm level and at an industry level, including sources of assistance during the recovery phase; and
 - d) social and other sources of support for producers coping with this sort of problem.

11.2 Tracking Oyster Movements

Major recommendations regarding ways to better understand and improve the tracking of Oyster movements include:

- 6. Complete a Gap Analysis of state tracking systems and make recommendations for improvements.
- 7. Undertake a Gap Analysis and assessment of the need for state systems to be linked to a national tracking system.
- 8. Undertake an immediate risk assessment on likely vectors of transferring the virus within a state and between states.

11.3 Monitoring POMS

Recommendations to improve communication and achieve an on-going and adequate oyster mortality monitoring system in each state include:

- 9. Recommendations developed jointly by industry and government regarding appropriate level of *active* monitoring and surveillance for POMS.
- 10. Industry and Government communication and extension strategies developed to address key issues (eg to ensure growers know correct % of mortalities that requires reporting).
- 11. Industry and government to work together to encourage industry to report mortality events when appropriate.
- 12. Investigate the use of sentinel populations in high risk areas of potential viral infection. This may involve a mixture of cultivated and feral oyster populations.
- 13. Develop national capacity and capability to report and monitor non-harvest stock movements between states and within each state.

11.4 Hatchery Protocols

Major recommendations regarding hatcheries and hatchery protocols include:

- 14. Undertake a Gap analysis regarding the need for national testing protocols for hatcheries.
- 15. Investigate the development of national hatchery protocols regarding testing for POMS if demonstrated by Gap analysis.
- 16. Increase strategic extension to industry regarding emergency response scenarios and monitoring protocols for hatcheries.

The recommendations above are a key outcome of the project and should greatly assist industry and government to act in a coordinated and integrated way forward regarding Australia's response to the imposing threat and reality of the Pacific Oyster Mortality Syndrome.

(Note: additional industry recommendations and key research priorities identified in the <u>French Study Tour Report</u> are included within Appendix 3).

12 References

Cairns 2011 – *Final Report OsHV-1 \muVar International Workshop*, available from: <u>http://www.oysterstasmania.org/downloads/Oyster-Herpes-Virus-Workshop-Final-Report-111107.pdf</u> and also included in Appendix 3.

Understanding and planning for the potential impacts of OsHV-1 μ Var on the Australian Pacific oyster industry: French Study Tour Report, available in Appendix 3 and from: <u>http://www.oysterstasmania.org/news/frdc-poms-project-study-tour-to-france-final-report</u>

POMS Question and Answer Fact Sheets (short & long versions), available in Appendix 3 and from: <u>http://www.oysterstasmania.org/news/poms-question-a-answer-fact-sheets-now-available</u>

Appendix 1: Intellectual Property

The research is for the public domain. The report and any resulting manuscripts are intended for wide dissemination and promotion. All data and statistics presented conform to confidentiality arrangements.

Appendix 2: Staff

Staff and individuals involved in and consulted during this project include:

Bruce Zippel: Chair, Oysters Australia, Chair, South Australian Oyster Growers Association James Calvert: Oysters Australia, Tasmanian Shellfish Executive Council Tony Troupe: Oysters Australia, NSW Oyster Growers Association Angus Cameron: AusVet Animal Health Services Rob Moxham: NSW grower Daniel Webb: Tasmania Grower Steve Bowley: Chair, SA Oyster Research Council Steven Jones: Aglign atf Mattamatta Oysters Trust Scott Parkinson: Shellfish Culture Ltd Ian Duthie: Chair, Tasmanian Oyster Research Council Ben Cameron: Cameron of Tasmania Pty Ltd Cultured Shellfish Kerry Wells: Shellfish Culture Tasmania Kevin McAsh: McAsh Oysters Jill Coates: SA Oyster Growers Association Executive Wayne O'Connor: NSW DPI, Port Stephens Fisheries Institute Jane Francis: NSW DPI Mark Crane: Program leader, FRDC Animal Health subprogram Kevin Ellard: DPIPWE, Tasmania Shane Roberts: PIRSA Fisheries and Aquaculture Trudy McGowan: South Australia Oyster Growers Association Rachel King: Oysters Australia Ray Murphy: Oysters Tasmania National POMS Advisory Group Tom Lewis, RDS Partners Morag Anderson, RDS Partners Don Defenderfer, RDS Partners

Appendix 3: Current Position and Future Plans for the Australian Industry Understanding and planning for the potential impacts of OsHV-1 µVar on the Australian Pacific oyster industry (FRDC 2011/043)

Current position and future plans for the Australian industry

7 July 2012





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Acknowledgements

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The project was managed by Tom Lewis and Don Defenderfer at RDS Partners. This report and project were overseen and given key input by members of the Oysters Australia POMS Advisory Group including Bruce Zippel, James Calvert, Tony Troup, Scott Parkinson and Steve Bowley.

Individuals involved in and/or consulted during this project include: Bruce Zippel: Chair, Oysters Australia, Chair, South Australian Oyster Growers Association; James Calvert: Oysters Australia, Tasmanian Shellfish Executive Council; Tony Troupe: Oysters Australia, NSW Oyster Growers Association; Angus Cameron: AusVet Animal Health Services; Rob Moxham: NSW grower; Daniel Webb: Tasmania Grower; Steve Bowley: Chair, SA Oyster Research Council; Steven Jones: Aglign atf Mattamatta Oysters Trust; Scott Parkinson: Shellfish Culture Ltd; Ian Duthie: Chair, Tasmanian Oyster Research Council; Ben Cameron: Cameron of Tasmania Pty Ltd Cultured Shellfish; Kerry Wells: Shellfish Culture Tasmania Kevin McAsh: McAsh Oysters; Jill Coates: SA Oyster Growers Association Executive; Wayne O'Connor: NSW DPI, Port Stephens Fisheries Institute; Jane Francis: NSW DPI; Mark Crane: Program leader, FRDC Animal Health subprogram; Kevin Ellard: DPIPWE, Tasmania; Shane Roberts: PIRSA Fisheries and Aquaculture; Trudy McGowan: South Australia Oyster Growers Association; Rachel King: Oysters Australia; Ray Murphy: Oysters Tasmania; National POMS Advisory Group.

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Appendix 1: POMS Q & A Face Sheet: Short Version

- Appendix 2: POMS Q & A Fact Sheet: Long Version
- Appendix 3: Final Report of the International OsHV-1 µVar Workshop, Cairns July 2011
- Appendix 4: French Study Tour Report

Appendix 5: Briefing Note: Emergency Aquatic Animal Disease Preparedness in Tasmania in

response to detection of OsHV-1 μVar^* in New Zealand

1 Executive Summary

This report summarises current understandings and *opinions* and describes desired outcomes and actions regarding four key issue areas associated with the Pacific Oyster Mortality Syndrome (POMS), the name that has been given to the OsHV-1 μ Var mediated viral disease associated with high mortality events in the Pacific oyster (*Crassostrea gigas*). (The term POMS relates to mortalities of Pacific Oysters under varying environmental conditions and when infected by the virus. For the sake of this document, the use of the word POMS can also mean the presence of the virus.)

Four key emergency animal disease response issue areas frame this report:

- 1. Emergency Response Protocols
- 2. Tracking Oyster Movements
- 3. Monitoring POMS
- 4. Hatchery Protocols

This report also summarizes the recommendations made by the French Study Tour component of this project and gives an update on those recommendations, particularly on the research needs identified by the study group.

The document is based on research, interviews with key industry and government stakeholders and input from the Oysters Australia POMS advisory group.

Recommendations for action and strategic planning include:

1.1 Emergency Response Protocols

Key recommendations regarding emergence response protocols include:

- 1. Need to finalize membership and roles of national industry/government Emergency Response Group.
- 2. Government and industry to work together on emergency response scenarios and discuss on-going surveillance processes and Emergency Response Plans.
- 3. Increase communication between government and industry regarding Emergency Response protocols and requirements (eg clarification of % of mortalities that requires reporting in each state.)
- 4. Increase communication with New Zealand industry and government representatives regarding their emergency disease response processes and rebuilding strategies.
- 5. Develop a plan (or plans) for preparing OsHV-1 μ Var -focussed, industry-endorsed emergency response plan/s in SA, NSW and TAS. This plan (or plans) should include details of agreed:

- a) technical response options, including contingency planning and learning how to live with the disease and knowing what the options are for maintaining commercial production in an infected area;
- b) regulatory response options;
- c) financial options available at the individual farm level and at an industry level, including sources of assistance during the recovery phase; and
- d) social and other sources of support for producers coping with this sort of problem.

1.2 Tracking Oyster Movements

Major recommendations regarding ways to better understand and improve the tracking of Oyster movements include:

- 6. Complete a Gap Analysis of state tracking systems and make recommendations for improvements.
- 7. Undertake a Gap Analysis and assessment of the need for state systems to be linked to a national tracking system.
- 8. Undertake an immediate risk assessment on likely vectors of transferring the virus within a state and between states.

1.3 Monitoring POMS

Recommendations to improve communication and achieve an on-going and adequate oyster mortality monitoring system in each state include:

- 9. Recommendations developed jointly by industry and government regarding appropriate level of *active* monitoring and surveillance for POMS.
- 10. Industry and Government communication and extension strategies developed to address key issues (eg to ensure growers know correct % of mortalities that requires reporting).
- 11. Industry and government to work together to encourage industry to report mortality events when appropriate.
- 12. Investigate the use of sentinel populations in high risk areas of potential viral infection. This may involve a mixture of cultivated and feral oyster populations.
- 13. Develop national capacity and capability to report and monitor non-harvest stock movements between states and within each state.

1.4 Hatchery Protocols

Major recommendations regarding hatcheries and hatchery protocols include:

- 14. Undertake a Gap analysis regarding the need for national testing protocols for hatcheries.
- 15. Investigate the development of national hatchery protocols regarding testing for POMS if demonstrated by Gap analysis.
- 16. Increase strategic extension to industry regarding emergency response scenarios and monitoring protocols for hatcheries.

1.5 France Study Tour Report

Industry recommendations and key research priorities identified in the France Study Tour Report are detailed below (comments in italics identify the progress made against each action since the publication of the France report in December 2011). It is recommended that industry and government continue to act on these priorities.

- 17. Develop and implement plan for discussing tour findings with industry, researchers and regulators in SA, NSW and TAS.
 - Current status: Study tour findings were communicated to stakeholders via production and distribution of Study Tour Final Report (via email networks, industry web pages, webinar presentations and workshops).
- 18. Undertake an immediate risk assessment on likely vectors of transferring the virus within a state and between states.
 - Current status: Issues and recommendations associated with tracking oyster movements are incorporated into section 4.2 (Tracking Oyster Movements) below.
- 19. Investigate the use of sentinel populations in high risk areas of potential viral infection. This may involve a mixture of cultivated and feral oyster populations.
 - Current status: Monitoring of oyster movements, including issues and recommendations is discussed in section 4.3 (Monitoring POMS) below.
- 20. Develop national capacity and capability to report and monitor non-harvest stock movements between states and within each state.
 - Current status: Tracking oyster movements including issues and recommendations is discussed in section 4.2 below.
- 21. Develop a plan (or plans) for preparing an OsHV-1 μ Var focussed, industry-owned and coordinated emergency response plan in each of SA, NSW and TAS. This plan (or plans) should include details of agreed:
 - e) technical response options, including contingency planning and learning how to live with the disease and knowing what the options are for maintaining commercial production in an infected area
 - f) regulatory response options
 - g) financial options available at the individual farm level and at an industry level, including sources of assistance during the recovery phase
 - h) social and other sources of support for producers coping with this sort of problem
 - Current status: These actions are discussed and have been incorporated into section 4.1 (Emergency Response Protocols) below.

- 22. Increase selective breeding focus on developing virus resistant family lines that maintain the economic value already realised.
 - Retain commitment to the ASI Breeding Program, as this is the best vehicle available to breed for resistance to POMS whilst minimising and loss of economic importance. (ASI is industry owned, having the Tasmanian Oyster Research Council and South Australian Oyster Research Council as its shareholders.)
 - Ensure that the Review of ASI committed to by Oyster industry key stakeholders allows for and is funded by the CRC and ensures capacity to deliver family lines that are resistant to POMS.
 - Funding needed to ensure family lines are tested within laboratories using a developed infectivity model based on French experience.
 - Testing of lines developed through crossing tetraploids with ASI resistant family lines is needed (being done by Shellfish Culture and University of Sydney).
 - CRC investment via the Oyster Consortium into testing the performance of ASI Family Lines in the Georges River has been committed to.
- 23. Establish a trial in the Georges River NSW to test the effect of growing height and oyster density on mortalities (possibly 3 heights, 3 densities, 3 replicates = 27 baskets).
 - First round has been done by University of Sydney in the summer of 2011/2012.
 - Further work needed to verify last season's results.
- 24. Establish a series of trials in the Georges River (NSW) to test the effectiveness of other growing systems including adjustable longline systems and the floating basket system in use in the NSW oyster industry.
 - This has been funded as part of the FRDC POMS Project to be delivered by University of Sydney.
 - University Sydney has committed to undertake this work this coming summer, and will use floating systems and different variations of adjustable longline systems.
 - University of Sydney is working closely with industry to put systems in place, with some equipment being donated by Basked Manufacturer.
 - University of Sydney has addressed this research priority.
- 25. Adapt the French infectivity models as published by IFREMER in an Australian biosecure facility as the basis for direct research into different aspects of the virus.
 - This is a requirement of an FRDC-funded POMS project.
 - University of Sydney has agreed to do this, but working to ensure that Model is developed for use this calendar year (yet to be resolved).
 - Need to ensure that a model is available for industry use at reasonable rates.

- Funding needed for ASI to test family lines using the Infectivity Model.
- University of Sydney is in close contact with INFREMER Scientists who have developed the model.
- 26. Standardised protocol for PCR testing for the virus within Australia to provide confidence in result comparison between testing agencies.
 - Part of current FRDC POMS project, but likely to be in the latter component of the project.
 - General Agreement amongst the SCAAH group for this, but no real drivers to sign off on it.
 - Need to make information available for those wanting to develop new PCR Analysis.
 - Work with SCAAH to push through Standardisation as per FAO listing (put together by Trystan from IFREMER).
- 27. Run a temperature "stress" trial to establish if increasing the culture temperature by about 1C per day to above 17C will elicit disease in sub-clinically infected oysters (if successful, this would be used as a fast and cheap test for the presence of virus in oysters).
 - A component of an FRDC-supported POMS Project, but not specific.
 - Probably need a further recommendation here to push this through as part of the studies undertaken using the infectivity model.
 - Enter into discussions with University of Sydney or other Research Providers to deliver on this one.
 - Perhaps include SCAAH in discussions to ensure compatibility with further testing for POMS.
- 28. Research the ability of other bivalve species to act as translocation and/or disease vectors.
 - Part of POMS project done by University of Sydney
 - *Probably later in the project.*
 - Industry to liaise and encourage existing research
- 29. Determine whether vertical transmission of the virus occurs.
 - Same as above points exactly.
- 30. Establish if virus has spread (e.g. north and south of Sydney Harbour).
 - Not really part of the POMS project, perhaps seek Department of Primary Industries in NSW Support to take samples and have them tested.

2 Background

This project was initiated through discussions between the FRDC and the Shellfish Industry Council of Australia in response to the threat posed to the Australian Pacific oyster industry by the incursion of the POMS virus - which has been responsible since at least 2008 for devastating mortalities of 80-100% of farmed oysters in parts of Europe, France and New Zealand.

In 2010 the virus was identified in New Zealand where it has had major impacts upon the oyster industry; in late 2010 the disease was found in dead and dying Pacific oysters in an estuary in NSW. In early 2011 the disease was found in a second estuary in NSW. The virus is now listed as notifiable disease.

In 2011 Australian industry, scientists and regulators recognised the massive threat that this virus poses to the Australian Pacific oyster farming industry. The urgent need for a national strategy to understand, control and/or mitigate the effects of this virus was prompted by industry, scientists and regulators and a national POMS Advisory Group was formed to guide a strategic way forward.

FRDC funding was received in July 2011 for a project to help industry understand the disease so as to be able to mitigate and prevent its spread to other oyster growing regions in Australia. The project had three main objectives:

- A desktop study on industry relevant issues associated with the OsHV-1 μ Var virus and the related Pacific Oyster Mortality Syndrome (POMS).
- A field visit to France by a small group of industry representatives to discuss firsthand the French industry and regulatory experience regarding the effects and management of OsHV-1 μVar.
- The development and extension of a national strategy to control and/or minimise the spread of OsHV-1 μ Var in Australia and to develop management strategies to mitigate the effects of the disease in areas in which is, or may become, established.

The project's first stage, the desktop analysis and subsequent development of extension materials (see POMS Q & A Fact Sheets - short and long versions - in Appendix 1 and 2 and <u>on line</u>) were significantly informed by the deliberations and final report of the International OsHV-1 μ Var workshop that followed the Aquatic Animal Health Conference in Cairns in July 2011 (see Appendix 3 and <u>on line</u>).

The second stage of the project, the study tour to France, significantly increased the understanding of the oyster industry about the adverse affects of POMS and the strategies – including extension, research and development – that are needed if we hope to successfully combat the virus in Australia. The report of the France Study Tour is available <u>on line</u> as well as in Appendix 4 and significantly informs this report.

Following the study tour, the project's third objective was amended slightly to focus more on pragmatic industry needs.

The third stage of this project, the development of a POMS industry response plan (this report), focuses on the potential movement of the virus into other Pacific oyster production areas around Australia and the need for a strategic approach. This report summarises current information and desired outcomes and actions – discussing current understandings and *opinions* regarding POMS by industry and government and making recommendations regarding four key industry issues associated with the virus:

- A. Emergency Response Protocols
- B. Tracking Oyster Movements
- C. Monitoring POMS
- D. Hatchery Protocols

There has been extensive consultation with growers from major Pacific oyster-producing states, DAFF and AAHL during the development of this project and the identification of the four key issues identified.

It is recognized that the final recommendations will need to be accepted and endorsed by appropriate industry, science and regulatory agencies before the next phase of POMS investment, extension and research can commence. It is also recognized that a considerable amount of research and progress towards strategically *managing* POMS has been undertaken (and is the process of being undertaken) since the original project application was developed in 2011.

3 France Study Tour

Following the 2010 confirmation of Pacific oyster mortalities in New Zealand and NSW associated with the presence of the OsHV-1 μ Var virus, the Australian oyster industry and FRDC supported¹ an industry study tour to France to gain first hand information of the effect on the French industry of the virus and the response to this threat by industry, researchers and regulators.

The study tour team comprised:

- Growers: Bruce Zippel, Rob Moxham, James Calvert;
- Epidemiological expertise, cultural attaché, translation and tour logistics: Angus Cameron, Cate Mackenzie (<u>AusVet Animal Health Services</u>); and,
- Project manager: Tom Lewis.

Between 1 and 10 November 2011, the study team travelled from Paris to Normandy, around the French coast to the Mediterranean and back to Paris, meeting with growers, processors, industry representatives, researchers and government agencies.

A daily "blog" for the study tour (<u>www.oystertour.wordpress.com</u>) was maintained to provide information in real time to interested parties and to enable them to provide feedback and ask questions during the tour.

The blog remains online as a resource for stakeholders, to add background to the contents of this report and to provide a summary of the study team's thinking at the end of the tour. The French study tour report identified a number of industry actions and research priorities that inform the context, knowledge and recommendations of this document. The National POMS industry working group has endorsed these recommendations (particularly the research priorities).

Industry actions identified in the France study tour report are listed below. Comments in italics identify the progress made against each action since the production of the France report in December 2011.

- 1. Develop and implement a plan for discussing tour findings with industry, researchers and regulators in SA, NSW and TAS.
 - Current status: Study tour findings were communicated to stakeholders via production and distribution of Study Tour Final Report (via email networks, industry web pages, webinar presentations and workshops).

¹ James Calvert's participation was funded by Tas Prime Oysters. All others were supported through a combination of FRDC, Tasmania, SA and NSW oyster industry research council contributions.

- 2. Undertake an immediate risk assessment on likely vectors of transferring the virus within a state and between states.
 - Current status: Issues and recommendations associated with tracking oyster movements are incorporated into section 4.2 (Tracking Oyster Movements) below.
- 3. Investigate the use of sentinel populations in high risk areas of potential viral infection. This may involve a mixture of cultivated and feral oyster populations.
 - Current status: Monitoring of oyster movements, including issues and recommendations is discussed in section 4.3 (Monitoring POMS) below.
- 4. Develop national capacity and capability to report and monitor non-harvest stock movements between states and within each state.
 - Current status: Tracking oyster movements including issues and recommendations is discussed in section 4.2.
- 5. Develop a plan (or plans) for preparing an OsHV-1 μ Var focussed, industry-endorsed and coordinated emergency response plan in each of SA, NSW and TAS. This plan (or plans) should include details of agreed:
 - i) technical response options, including contingency planning and learning how to live with the disease and knowing what the options are for maintaining commercial production in an infected area
 - j) regulatory response options
 - k) financial options available at the individual farm level and at an industry level, including sources of assistance during the recovery phase
 - social and other sources of support for producers coping with this sort of problem
 - Current status: These actions are discussed and have been incorporated into section 4.1 (Emergency Response Protocols) below.
- 6. Increase selective breeding focus on developing virus resistant family lines that maintain the economic value already realised.
 - Retain commitment to the ASI Breeding Program, as this is the best vehicle available to breed for resistance to POMS whilst minimising and loss of economic importance. (ASI is industry owned, having the Tasmanian Oyster Research Council and South Australian Oyster Research Council as its shareholders.)
 - Ensure that the Review of ASI committed to by Oyster industry key stakeholders allows for and is funded by the CRC and ensures capacity to deliver family lines that are resistant to POMS.
 - Funding needed to ensure family lines are tested within laboratories using a developed infectivity model based on French experience.

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- CRC investment via the Oyster Consortium into testing the performance of ASI Family Lines in the Georges River has been committed to.
- 7. Establish a trial in the Georges River NSW to test the effect of growing height and oyster density on mortalities (possibly 3 heights, 3 densities, 3 replicates = 27 baskets).
 - First round has been done by University of Sydney in the summer of 2011/2012.
 - Further work needed to verify last season's results.
- 8. Establish a series of trials in the Georges River (NSW) to test the effectiveness of other growing systems including adjustable longline systems and the floating basket system in use in the NSW oyster industry.
 - This has been funded as part of the FRDC POMS Project to be delivered by University of Sydney.
 - University Sydney has committed to undertake this work this coming summer, and will use floating systems and different variations of adjustable longline systems.
 - University of Sydney is working closely with industry to put systems in place, with some equipment being donated by Basked Manufacturer.
 - University of Sydney has addressed this research priority.
- 9. Adapt the French infectivity models as published by IFREMER in an Australian biosecure facility as the basis for direct research into different aspects of the virus.
 - This is a requirement of current FRDC POMS project.
 - University of Sydney has agreed to do this, but working to ensure that Model is developed for use this calendar year (yet to be resolved).
 - Need to ensure that a model is available for industry use at reasonable rates.
 - Funding needed for ASI to test family lines using the Infectivity Model.
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 - Perhaps include SCAAH in discussions to ensure compatibility with further testing for POMS
- 12. Research the ability of other bivalve species to act as translocation and/or disease vectors.
 - Part of POMS project done by University of Sydney
 - Probably later in the project.
 - Industry to liaise and encourage existing research
- 13. Determine whether vertical transmission of the virus occurs.
 - Same as above points exactly.
- 14. Establish if virus has spread (e.g. north and south of Sydney Harbour).
 - Not really part of the POMS project, perhaps seek Department of Primary Industries in NSW Support to take samples and have them tested.

4 Current information, desired outcomes and recommended actions

This section summarises current understandings and *opinions* and describes desired outcomes and recommended actions regarding four key issue areas (4.1 Emergency Response Protocols, 4.2 Tracking Oyster Movements, 4.3 Monitoring POMS and 4.4 Hatchery Protocols) associated with POMS. Key industry and government perspectives are presented for each issue from each state.

4.1 Emergency Response Protocols

4.1.1 Industry perspectives

4.1.1.1 Tasmania – Current Situation

- Tasmanian Oyster industry has well established communication procedures provided by Oysters Tasmania, an initiative of both TSEC and TORC. Oysters Tasmania has contact details of all growers, a website and e-newsletter and direct email/phone contact.
- POMS brochures have been distributed widely through industry.
- There is increasing awareness of translocation implications by industry.
- Industry is continuing to be proactive to further promote POMS related issues, especially monitoring and reporting of mortalities.
- Industry has a partnership with the State Government for the Tasmanian Pacific Oyster Health Surveillance Program. The project aims to achieve the following objectives and monitoring for OsHV-1 μ Var is to be incorporated into this on-going program.
- A Briefing Note was prepared for industry by DPIPWE re Emergency Aquatic Animal Disease Preparedness in Tasmania in response to detection of OsHV-1 μVar in New Zealand – see Appendix 5.
- Tasmania has a large pool of people trained to work in an emergency animal disease response. (DPIPWE currently has about 120 people on standby. If a response to an outbreak of OsHV-1 μ Var were similar in scale to the response Tasmania mounted to the abalone disease outbreak in 2008, the disease control centre would involve around 25 trained people together with a further 15 field personnel.)
- Tasmania has a well-developed emergency response plan that complements the various AQUAVETPLAN emergency aquatic animal disease response plans agreed nationally. (Emergency animal disease plans were activated in Tasmania for both equine influenza in 2007 and the abalone herpes virus in 2008.)
- Laboratory support for diagnostic testing required during any OsHV-1 μ Var emergency response would be provided by the DPIPWE Animal Health Laboratories and the CSIRO Australian Animal Health Laboratories in Geelong.

4.1.1.2 Tasmania – Future Needs

- Need for trained industry liaison officers.
- There is a need for the Tasmanian industry, with the assistance of DPIPWE, to develop a basic emergency animal disease response plan. (Such a plan would identify, in advance of any outbreak, **who** would have the authority to negotiate with government over the response, **who** would be the industry spokesperson and **what** means would be used to get vital information out to members quickly in the

event of an outbreak.)

- An industry survey is needed to identify current levels of mortality, along with the timing and conditions surrounding these mortalities.
- Following on from the implementation of the survey identified above, there is a need to coordinate a workshop between relevant DPIPWE personnel and oyster growers; the objective of this workshop would be to improve communication and understanding of what information is necessary to make a conclusive diagnostic determination.
- Review cost sharing arrangements. (There is no cost sharing agreement currently in place within Australia for any aquatic animal diseases and as a result no compensation available for any direct or contingent losses to individual producers and allied businesses arising from either an outbreak or response. Similarly, there would be no cost sharing available between the Tasmanian State and Federal governments to cover the cost of response measures, as occurs in terrestrial livestock industries.)

4.1.1.3 South Australia – Current Situation

- There is good communication between industry and government.
- POMS flyers have gone out widely (eg 100 have been laminated and sent out to growers with recent newsletter).
- Processes are in place: if an outbreak were to occur SAOGA would communicate with OA and determine who needs to be on the group to respond.
- Reporting mortalities is required above 25%. (Growers are more willing to report now there used to be a feeling it reflected their management skills.)

4.1.1.4 South Australia – Future Needs

- There will be a major workshop in August where growers will be workshopping emergency response scenarios to outbreaks, and then they will communicate/put in place state based protocols. They will also discuss husbandry techniques.
- SA workshop coming up will be an important opportunity to highlight the issues and keep informing people.
- There are multiple opportunities to learn from New Zealand's preparedness and response to POMS and current research being undertaken there; however greater effort must be made to ensure that learning opportunities continue to occur and two-way communication is maintained.

4.1.1.5 NSW – Current Situation

- Industry has a good relationship with government.
- Industry knows about the issues and is responding well.
- NSW is slowly moving towards an electronic tracking system.

4.1.1.6 NSW – Future Needs

- From a national perspective, need better communication with national biosecurity people need to have the national industry/government group finalized.
- Standard protocols for hatcheries would be good.
- Need to *look at* a national electronic system to track movements.

4.1.2 Government perspectives

4.1.2.1 Tasmania

- Emergency response processes are established but can always be improved. (Appendix 5 includes a useful Briefing Note that was prepared for industry by DPIPWE regarding Emergency Aquatic Animal Disease Preparedness in Tasmania in response to the detection of OsHV-1 µVar in New Zealand.)
- Reporting of mortalities is a license condition.
- Government is happy to work more with industry on emergency response scenarios/plans and discuss on-going surveillance processes.
- Government will continue to liaise with industry's representative body regarding a mutual understanding of what would happen in an emergency.
- Government is happy to work with industry and further discuss Emergency Response protocols.
- Industry needs to continue to continue to communicate with government and know who to contact – government not confident all growers know who to contact in an outbreak or emergency.
- One of the biggest problems with managing oysters is farmers often don't know there's mortalities until it's too late. Need early detection systems.
- Industry and government can get more out of existing surveillance program now farmers need to notify government earlier about mortality events.
- Need to strengthen surveillance.
- Government would like to support what industry comes up with but have to keep in mind government resource limitations.

4.1.2.2 New South Wales

- Communication with industry is good.
- We have good industry champions.
- Have peak advisory group, newsletters, field days etc.
- Have biosecurity consultation group.
- Have cost sharing arrangements with industry now.
- Doing ER training and codes of practice with industry.

4.1.2.3 South Australia

- ER Plans and protocols are a high priority.
- Government is going to use POMS as an ER exercise.
- Industry/government communication has been good; positive messages being communicated.
- ERP are pretty high on industry's radar.

4.1.3 Summary - Key Government and Industry Issues:

- Government needs to continue to liaise with industry's representative body to ensure a mutual understanding of what would happen in an emergency.
- Government needs to work more with industry on emergency response scenarios and discuss on-going surveillance processes and EM plans.
- Reporting mortalities requirements still not clear to all growers eg % of mortalities that requires reporting.

- Industry is trying to get growers to be more responsible for reporting mortalities; some growers still not proactive when it comes to responding to mortality events and reporting to government as required.
- Need to have the national industry/government group finalized.
- State protocols regarding emergency response for hatcheries needs more communication/attention.
- Need to be proactive to continue learning from the New Zealand response to POMS, and current research and rebuilding processes.
- Establish formal communication linkage between the NZ researchers and industry response and Australian researchers and Industry coordinators.
- Investigate formalized agreement issues within Australia regarding aquatic animal disease emergencies and related issues.

4.1.4 Desired Outcomes: Emergency Response Protocols

- An emergency response system that is understood and agreed upon by industry and government.
- A practical emergency response system that allows government and industry to respond together to emergency circumstances in a fast, effective and integrated way

4.1.5 Recommended Actions: Emergency Response Protocols

- Finalize membership and roles of national industry/government ER group.
- Government and industry to work together on emergency response scenarios and discuss on-going surveillance processes and EM plans.
- Increase communication between government and industry regarding ER protocols and requirements (eg % of mortalities that requires reporting.)
- Increase communication with New Zealand industry and government representatives regarding their emergency disease response processes and rebuilding strategies.
- Develop a plan (or plans) for preparing an OsHV-1 μVar focussed, industry-endorsed and coordinated emergency response plan in each of SA, NSW and TAS. The plan (or plans) should include details of agreed:
 - technical response options, including contingency planning and learning how to live with the disease and knowing what the options are for maintaining commercial production in an infected area;
 - b) regulatory response options;
 - c) financial options available at the individual farm level and at an industry level, including sources of assistance during the recovery phase; and
 - d) social and other sources of support for producers coping with outbreaks, closures etc.

4.2 Tracking Oyster Movements:

4.2.1 Tasmania

• There is a need to identify typical oyster movement patterns within the state through an industry survey; this information could then be used to inform risk assessment and mitigation strategies.

- Nationally, movements of spat sales are tracked via established permit systems between states. Tasmania keeps track of imports through a permit system to maintain state quarantine requirements.
- It should be noted that as a licence condition for Pacific oyster growers in Tasmania, they must keep records of all fish brought onto and taken off the area to which this licence relates. However there is no requirement to report any movements to the government.

4.2.2 New South Wales

- NSW has tracking monitoring system within and between states. Movements mandatorily logged and reported.
- Government is looking at developing a voice recognition system reporting is a paper system now which is slower and prone to mistakes. Trialling electronic system in next two months.
- Tracking system in NSW is good but other states need sorting out.
- National system would be useful, re emergency preparedness.

4.2.3 South Australia

- Legislation covers monitoring what comes into the state (eg 80% from Tasmania) but no legislation requirement to report oyster movements within state. Protocols follow quarantine and pests requirements etc. (NSW denied application to bring in spat re POMS.)
- National system? Current systems ticking along. Something to look at.

4.2.4 Summary – State and National Issues:

- Limited state tracking systems in SA and Tasmania re movements within states and out of states. No real momentum for such systems. (NSW has state tracking system and is trialling electronic methods to improve paper system.)
- Tasmanian view: need to look at very closely where the bulk of the movements around the state happen so as to get a view of the general patterns of movement.
- National system would be useful, re emergency preparedness, but NSW government is the only state discussing it.

4.2.5 Desired Outcomes: Tracking Oyster Movements

- State and National tracking systems which provide sufficient information on oyster movements between and within states so that government and industry are able to respond quickly and appropriately to emergency disease outbreaks.
- Confidence by industry and government that the general pattern of oyster movements is understood adequately and that this level of understanding would be useful in the event of an emergency.

4.2.6 Recommended Actions: Tracking Oyster Movements

- Gap Analysis of state tracking systems and recommendations for improvements.
- Gap Analysis and assessment of need for state systems to be linked to a national tracking system.
- Undertake an immediate risk assessment on likely vectors of transferring the virus within a state and between states.

4.3 Monitoring POMS:

4.3.1 Tasmania – Current Situation

- State monitoring surveillance system is still going on.
- System could be improved.
- Challenges: acceptance by farmers (some still don't see it as important or don't want to deal with government).
- Monitoring 'could be our best investment' (i.e. sentinels) but national monitoring as was done last year is very resource intensive.

4.3.2 New South Wales – Current Situation

- Industry being proactive is a good thing industry can learn from terrestrial industries, re: dealing with diseases and reporting.
- Reporting of unexplained mortalities by industry can be done better.
- Government is always happy to hear from industry about losses government is interested whenever industry is concerned.
- Developing a national standard.
- National monitoring is not happening did major surveillance last year.
- Passive surveillance happens now. Using Fishcare volunteers, networks for passive surveillance.
- Industry is showing leadership from OA and NSW industry. Communication and coordination are good between government and industry.

4.3.3 South Australia – Current Situation

- Biggest issue is non-reporting of mortalities and diseases. They need to report unusual mortalities eg above 20%.
- Farmers don't necessarily regularly check and it's hard to tell diseases until the oysters are dying or dead. Mortalities highly variable between bays/farms.
- Need to improve reporting definition. (Note: SARDI researcher currently pulling together different definitions).
- Government likes early detection of diseases. Government working with industry but industry needs to take the lead regarding reporting if they don't report an event it can be devastating to their industry.

4.3.4 Summary – State and National Issues:

- Biggest issue is non-reporting of mortalities and diseases. Reporting of unexplained mortalities by industry can be done better.
- Challenges: acceptance by farmers (many still don't see it as important or don't want to deal with government)
- Monitoring could be effective investment (i.e. sentinels) but national monitoring as was done last year is very resource intensive.
- Mostly passive surveillance operating now.

4.3.5 Desired Outcomes: Monitoring POMS

- An on-going and adequate oyster mortality monitoring system in each state.
- National communication regarding results of state oyster monitoring system.

• Reporting of *unusual* mortality events adopted by industry as a standard operating procedure.

4.3.6 Recommended Actions: Monitoring POMS

- Recommendations developed regarding appropriate level of *active* monitoring and surveillance for POMS.
- Industry and Government communication and extension strategies (eg to ensure growers know correct % of mortalities that requires reporting).
- Industry and government to work together to encourage industry to report mortality events when appropriate.
- Investigate the use of sentinel populations in high risk areas of potential viral infection. This may involve a mixture of cultivated and feral oyster populations.
- Develop national capacity and capability to report and monitor non-harvest stock movements between states and within each state.

4.4 Hatchery Protocols

4.4.1 Industry Perspective:

- Protocols for testing in all hatcheries around Australia (details would need to be worked out with technical experts) should be developed.
- National standard testing across Australian hatcheries needed (questions such as who pays and who delivers it would need to be resolved).
- Need discussions with CVOs re protocols for testing. Need standard national approach (e.g. one hatchery in Tasmania has a strategy to test all oysters above 2.2 mm size).
- Need to focus on Early Detection and Translocation protocols (eg how can we legally stop gear movements and product movements quickly?)
- Need greater understanding of oyster movements: it is a 'spider web of movements if you were to track it' for example hatcheries put stock in every major oyster growing bay in Tasmania and SA every week.
- Need working group with hatchery representatives (eg major two major hatcheries) and CVOs.
- Hatcheries should be part of the Pacific Oyster Health Surveillance program eg testing twice a year.
- If infection occurs CVO will make recommendations so advice needed about what this would mean for hatcheries on a case by case basis eg details such as water release.
- There is a general lack of understanding about what would happen in a hatchery if there was an outbreak. Hatcheries need to know what they should do, or what would they be required to do. 'No one knows what would happen in an emergency', eg containment, how to stop it spreading to the next region. A national approach needed.
- Hatcheries have questions such as: what are the different scenarios and implications; what steps would be put in play and who is going to control it; how will industry survive it; and how would hatcheries/industry survive without supply?
- Need communication about protocols; need discussions with CVOs.
- Need working group to make it happen.

- If there is a problem it will probably be noticed in hatcheries first larvae and juveniles may be most vulnerable.
- Testing programs like the one that was undertaken becomes quickly redundant what's the incubation period for the virus? if longer than 48 hrs than that kind of testing wont' really help.

4.4.2 Summary - Hatchery Protocol Issues:

- Protocols (and national standards) for testing in all hatcheries around Australia needed (details would need to be worked out with technical experts).
- There is a general lack of understanding about what would happen in a hatchery if there was an outbreak. Hatcheries need to know what they should do, or what would they be required to do. Lack of knowledge about would happen in an emergency, eg containment, how to stop it spreading to the next region.
- A national approach to hatchery related issues is needed.
- Need greater understanding of oyster movements originating from hatcheries.

4.4.3 Desired Outcomes: Hatchery Protocols

- Government and industry confident that all hatcheries are adequately monitoring for POMS.
- A coordinated and integrated approach regarding the development of national hatchery protocols regarding testing for POMS.
- All hatcheries fully understanding emergency response protocols and procedures.
- A practical understanding of oyster movements originating from hatcheries and disease implications.

4.4.4 Recommended Actions: Hatchery Protocols

- Gap analysis for the need for national testing protocols for hatcheries.
- The development of national hatchery protocols regarding testing for POMS if demonstrated by Gap analysis.
- Extension to industry regarding emergency response and monitoring protocols for hatcheries.

5 Conclusion

Section 3 of this report provides an extract of the recommendations identified in the France Study Tour Report (see Appendix 4). Section 4 of this report presents current understandings, desired outcomes and recommended actions regarding the four key issue areas (4.1 Emergency Response Protocols, 4.2 Tracking Oyster Movements, 4.3 Monitoring POMS and 4.4 Hatchery Protocols) associated with POMS.

All of the key recommendations from Section 3 and 4 are summarized in the Executive Summary (Section 1) and are thus not repeated here.

As noted previously (Section 1), it is recognized that final recommendations will need to be accepted and endorsed by appropriate industry, science and regulatory agencies before the next phase of POMS investment, extension and research can commence. Any endorsement of those recommendations by non-industry groups would require subsequent process.

It is also recognized that a considerable amount of research and progress towards strategically *managing* POMS has been undertaken (and is the process of being undertaken) since the original project application was developed in 2011.

6 Acronyms

Appendix 1:

POMS Q & A Fact Sheet: Short Version

What is **POMS**....?

Pacific Oyster Mortality Syndrome (POMS) is the name that has been given to the viral disease associated with high mortality events in the Pacific oyster (*Crassostrea gigas*) in Europe, New Zealand and NSW.

POMS is serious.

POMS represents a major threat to the ongoing viability of the oyster industry. POMS has resulted in complete loss of farm stock in some areas together with restriction of oyster movements.

Is POMS a human health risk?

There is no evidence that the virus can infect humans

How is the virus spread?

Spread is most likely to occur through the movement of live oysters to uninfected areas, although spread by movement of equipment is also possible.



What should I do if I have a mortality event on my farm?

- **1.** STOP all movement of oysters, associated gear and equipment IMMEDIATELY.
- 2. REPORT all high or unexplained mortalities on your farm. This is a licencing condition of your industry. Reporting is a LEGAL REQUIREMENT.
- 3. Assume it's POMS and then ACT to safeguard your industry.
 24 hour disease hotlines have been established in each state.

SEE OVER FOR MORE DETAILS.

How can the spread of the virus be controlled?

Once detected, STOP all oyster and infastructure movement, and Seek advice IMMEDIATELY.

Farmer reporting of mortalities is the main system used to identify new outbreaks & greatly assists in controlling the spread of POMS.

REPORT all mortalities to POMS 24hr Hotline. **SEE OVER FOR DETAILS.**

POMS Reporting & Information Hotlines

South Australia Tasmania NSW 1800 675 888 (Open 24 HRS) 1800 065 522 1800 043 536 **DPIPWE General Enquiries:** 1300 368 550 Á Á http://www.dpi.nsw.gov. http://www.dpi.nsw.gov.au/ http://www.dpi.nsw.gov.au/ fisheries/aquaculture/info/ au/fisheries/aquaculture/ fisheries/aquaculture/info/ info/poms poms poms **Oysters Tasmania** 0458 601 057 **Oysters South Australia** 0407 883 333 www.oysterstasmania.org www.oysterssa.com.au RDS Partners Australian Government

Fisheries Research and Development Corporation

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Appendix 2:

POMS Q & A Fact Sheet: Long Version



Pacific Oyster Mortality Syndrome:



Australian Government

Fisheries Research and Development Corporation

Question & Answer Fact Sheet

1. What is POMS?

(Long Version)

Pacific Oyster Mortality Syndrome (POMS) is the name that has been given to Pacific oyster mortalities associated with the virus Ostreid herpesvirus-1 microvariant (OsHV-1 μ Var) in Australia.

The virus has been associated with high mortality events (often brought on by environmental or handling stress) involving the Pacific oyster (*Crassostrea gigas*) in Europe, New Zealand and NSW.

All ages of Pacific Oysters may be affected, but spat and juvenile oysters often suffer higher mortalities. To date there is no evidence of POMS affecting any other oyster species.

2. How serious is the disease?

The disease is **very serious**; it has resulted in the complete loss of farm stock in some areas together with restriction of oyster movements.

Restrictions imposed on affected areas means that no oysters, parts of oysters or oyster equipment can be moved from these locations to other areas. Significant economic losses caused by mortalities have been experienced by affected farms.

There is a **potential high future impact** in Australia since the disease represents a major threat to the ongoing viability of the industry.

3. Where is the disease now?

In <u>France</u> higher mortalities were first reported in 2008 and have continued into 2011. The <u>UK</u>, Jersey, Ireland and the Netherlands have all suffered recent mortalities.

In <u>New Zealand</u> the disease was confirmed in late 2010. The virus appears to be widespread in the northern part of the North Island.

In Australia, mortalities occurred in two estuaries in <u>NSW</u> (Botany Bay and Port Jackson) in late 2010. After extensive testing in NSW, SA and Tasmania it appears to be limited to these two estuaries. Nearly all of the cultivated Pacific oysters in the Georges River (Botany Bay) have died.

4. Is POMS a human health risk?

There is no evidence that the virus can infect humans. The virus has only been reported to affect Pacific oysters and cannot be transmitted to humans.

There is no food safety or human health issues related to the <u>POMS event in NSW</u>. The NSW Food Authority assures consumers that the stringent safeguards in place under the <u>NSW Shellfish Program</u> ensures oysters destined for sale for human consumption from NSW are safe to eat.

5. How is the virus spread?

How the disease is spread is still not clear. Very little objective information is available about the major factors responsible for the outbreaks. It is *theorized* that international spread of the disease *may* have taken place in association with biofouling (e.g. oysters) attached to the hulls of ships.

Within France and New Zealand **spread is most likely to have occurred through the movement of live infected oysters to uninfected areas** (although spread by movement of equipment is also possible).

The virus is often inactive in cooler waters (below about 17°C). It is possible for oysters to be carrying the virus and not get sick until the water temperature rises or the oysters are subjected to environmental or handling stress.

Transmission over small distances is likely to occur through the movement of particles suspended in the water column.

6. What should I do if I have a mortality event on my farm?

The virus has been placed on the <u>national list</u> and is thus reportable in all states. Under state Acts, if there is suspicion of POMS on a farm it is required by law to be reported to government; 24 hour emergency disease hotlines for such purposes are detailed below.

Under license conditions, growers are required to report high or unexplained mortalities on their farms. Mortalities should be *immediately reported* so that testing can occur to identify the cause.

If you notice high oyster mortality, you should *immediately stop any movement of oysters* and associated gear and equipment. Get your oysters tested ASAP and follow industry and regulatory protocols.

Until the cause of any high mortality is identified, you should assume that it could be POMS and you should act accordingly to safeguard your industry.

7. How can its spread be controlled?

Farmer reporting of mortalities is the main system used to identify new outbreaks and greatly assists in controlling the spread of POMS. **Remember: Report any mortalities or risks immediately.**

If the disease is detected in an area, state restrictions regarding the movement of oysters, oyster farming materials and associated equipment are likely to be imposed until the full extent of the virus is ascertained.

Government veterinary laboratories can usually rule out POMS as the potential cause within a few days of receiving samples. Local state veterinary or fisheries agencies (see contacts below) will be able to assist with the submission of samples to relevant laboratories.

If the virus is detected, cease all translocations immediately amongst all growing areas in your state.

8. Should growers be monitoring for POMS?

A national surveillance program has taken place regarding POMS (the results of which will help with future management options for the industry), however all growers must be vigilant in looking out for oyster mortalities and must report any unusual events as soon as possible.

Advice is available on monitoring and surveillance for POMS (see industry contacts below).

9. What is being done to limit the spread of the disease?

Each state has established processes to assist industry in the prevention and spread of POMS. National monitoring has occurred and biosecurity and emergency response plans are established in each state.

Currently oyster and equipment movement restrictions apply within NSW; however these restrictions also affect oyster movement between states.

New regulations prohibit the importation of whole oysters into <u>Tasmania</u> from all states or territories; in addition, oysters in the half shell originating from NSW may not be brought into the state.

10. How should I prepare my business in the case that my oysters are affected by POMS?

Developing a POMS risk management plan for your business is recommended. Be prepared.

Your business should be prepared to answer contingency questions such as: if my farm is affected by POMS and severe restrictions on the movement of oysters from that area are imposed, what would this mean for my business?; what is my business plan to manage a POMS event and ensure my business's long term financial survival?; and where can I get risk management advice? See industry contacts below for assistance.

11. The future: what is being done to control the disease?

The oyster industry is actively employing and researching a number of strategies to manage and contain the disease to the two NSW bays where it is already present.

Management strategies and research projects currently include: breeding Pacific oysters for resistance; growing hatchery spat to a larger size before stocking; understanding how new husbandry methods can protect against mortalities; and emergency harvest in the face of possible outbreaks.

A group of Australian industry leaders <u>toured</u> oyster farms in France in November 2011 to better understand the impacts of the virus and how the Australian industry can proactively manage the disease.

12. For further information about POMS or to report oyster mortalities:

NSW:

Web:	NSW Farmers Association - Oysters	Ph:	02 8251 1700	
Web:	Pacific Oyster Mortality Syndrome – NSW			
Ph:	Oyster Mortality Reporting	Ph:	1800 043 536 or 02 4982 1232	
South Australia:				
Web:	Oysters South Australia	Ph:	0407 883 333	
Web:	Biosecurity South Australia	Ph:	1800 065 522	
Tasma	ania:			
Web:	Oysters Tasmania	Ph:	0458 601 057	
Web:	DPIPWE site relating to reporting of disease	Ph:	24 hour hotline: 1800 675 888	
	DPIPWE re import conditions for molluscs			

13. Other Key Links:

Report: Final Report OsHV-1 µVar International Workshop, Cairns, 9-10 July 2011

Slides: <u>Power point presentation: Pacific Oyster Mortality Syndrome</u>

Media: Oyster industry learning international lessons to stop virus spread

Appendix 3:

Final Report of the International OsHV-1 µVar Workshop,

Cairns July 2011



Australian Government Fisheries Research and Development Corporation



FINAL REPORT OsHV-1 µVar

Ostreid herpesvirus-1 µVar

INTERNATIONAL WORKSHOP

Cairns, Queensland Australia 9-10 July 2011

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The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

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1. SUMMARY OF MAJOR FINDINGS

THE DISEASE

- Ostreid herpesvirus-1 microvariant (OsHV-1 μVar) has been associated with high mortality events involving the Pacific oyster (*Crassostrea gigas*) in Europe, Australia and New Zealand.
 - In France, these higher mortalities started in 2008 and have continued in 2009 and 2010
 - UK, Jersey, Ireland and the Netherlands have all experienced mortalities
 - In Australia, mortalities occurred in late 2010 and, to date, appear to be limited to two estuaries
 - In New Zealand, mortalities may have started in early 2010 and OsHV-1 µVar was confirmed with a second round of mortalities in late 2010. The virus appears to be widespread in the northern part of the North Island.
- The disease is characterised by high mortalities in Pacific oysters. All ages may be affected, but spat and juvenile oysters often suffer higher mortalities. Other species may become infected but have not exhibited mortalities associated with the virus.
- There is no evidence that the virus can infect humans.

THE VIRUS

- The virus is characterised primarily by a 13 base-pair deletion in the C region of ORF4.
- Phylogenetic studies indicate that the New Zealand strain shared a 13bp deletion in the non-coding area of the C-region, although minor other point mutations in the coding area of the C-region were not present in the NZ isolate as compared with the French strain of OsHV-1 μ Var. The Japanese and Chinese isolates (despite absence of reports of large scale mortalities) are also more closely related to OsHV-1 μ Var than the reference strain of OsHV-1.
- The OsHV-1 µVar has completely replaced classical OsHV-1 as the dominant strain isolated during mortality events from oysters in France since mid-2008.

DIAGNOSIS

- o Increased mortality rate
- Histopathology is generally non-specific, but recent work indicates that it may be possible to identify typical lesions. Histological examination continues to play an important role to exclude other possible causes of mortalities such as infection with protozoan parasites.
- PCR is used for surveillance and for confirmation of suspect cases.
 - A variety of different PCR tests are being used, with different primers and formats.
 - This lack of standardisation may pose a potential problem. Current work to draft a chapter on OsHV-1 µVar for the OIE Manual of Diagnostic Tests for Aquatic Animals is likely to help resolve this lack of standardisation.
 - Existing tests have not been adequately validated.

TRANSMISSION AND SPREAD

- Transmission is horizontal and is likely to occur through the water body.
- There is some evidence from New Zealand that uninfected larvae may be able to be produced from infected brood stock.
- While sub-clinical infections may occur, it is not known if a true latent infection occurs.
- Spread is most likely through the movement of live oysters, although spread by movement of equipment is also possible.
- It was hypothesised that international spread may have taken place in association with biofouling (i.e. oysters attached to the hulls of ships).

SURVEILLANCE

- Passive farmer reporting is the main system used to identify new outbreaks in all countries.
 - Currently, on-farm mortality events occur relatively frequently for a variety of reasons. Many of these events are not reported.
 - Current reporting pathways are not clear to all growers
 - Collation, analysis and response may need improvement
 - An industry benchmarking project may offer a mechanism for improving passive reporting

- Structured surveys are underway in Australia and the UK to demonstrate freedom from infection in the non-affected areas.
- Few mechanisms exist to routinely collect risk factor data (other than data loggers for water temperature and water quality).

RISK FACTORS

- The disease is multifactorial. Factors that are likely to be associated with the disease include:
 - Host
 - Pacific oyster
 - Spat and juveniles appear to be more susceptible
 - Rapidly growing oysters may be more susceptible
 - Genetics: There is evidence of decreased mortalities in certain Pacific oyster families in challenge trials
 - Pathogen
 - OsHV-1 μ Var
 - Evidence, especially from France, of co-infection with other pathogens including *Vibrio* spp. and various parasites.
 - Environment
 - Water temperature: Outbreaks are rare below 16°C or 18°C
 - Water quality
 - Depth: Intertidal oysters may be less affected
 - Proximity to other outbreaks: Remote oysters are less affected.
- The role of the following risk factors is inconsistent:
 - Wild vs. hatchery spat
 - Triploid vs. diploid oysters
- The following factors appear to play no important role
 - Salinity

REGULATIONS

- Current control measures are based on restrictions of movements of oysters and, in some countries, infrastructure out of affected estuaries.
 - Legislative controls in different countries vary, as do the strength of the control measures.

- OsHV-1 μVar appears to meet the criteria for being listed by the OIE, but no request for listing has yet been made by an OIE member state.
 - Listing would provide increased information about the global distribution of the virus, help limit the spread, and may assist in obtaining funding for research and surveillance. On the other hand, listing would limit exports from affected countries.

CONTROL MEASURES

- Strategies currently being used, or under research, for controlling the disease when it is already present include:
 - Breeding Pacific oysters for resistance
 - Use of alternative, resistant species
 - Growing hatchery spat to a larger size before stocking
 - Avoidance of stocking susceptible animals during periods of warmer water temperature
 - Stocking larger numbers of spat to compensate for the expected losses
 - Emergency harvest in the face of possible outbreaks

2. RECOMMENDATIONS

COORDINATION AND COLLABORATION

- 1. Establish an international OsHV-1 μVar expert **technical advisory and coordination group** to promote and coordinate collaborative work in the following areas:
 - Phylogenetic studies
 - Histology
 - Test validation
 - Global surveillance

It is proposed that the FRDC Aquatic Animal Health Subprogram (AAHS) may initially form the basis of this group. In order for AAHS to take on the role of the technical advisory and coordination group it may wish to consider:

- Industry participation to include oyster industry representative/s
- Inclusion of the Australian state jurisdictions involved in oyster production

- Establishment of international links and collaboration, in particular with IFREMER, OIE Reference Laboratories, and industry and researchers from not only affected countries but also unaffected countries with large oyster industries.
- Pursuing active engagement with **Asian industry and researchers** to promote collaborative research and sharing of surveillance information. Asia has the world's largest Pacific oyster industries. Sharing any experience Asian producers may have with similar diseases, and collaborating with them to develop measures for early detection and control, would be mutually beneficial.
- Actively engaging major oyster-producing countries (affected and unaffected) in collaborative research and collaborative funding of research. Unaffected major oyster-producing countries (such as, presumably, China, Japan, Korea and the USA) have a vested interest in improving our understanding of OsHV-1 µVar in order to improve quarantine, preparedness and rapid response capabilities. Funding offshore research may be seen as an excellent investment.
- Membership should therefore represent the following groups (from both within Australia and internationally):
 - Laboratory scientists
 - o Epidemiologists
 - o Regulators from national and state jurisdictions
 - o Farmers
 - o Hatchery managers
- 2. **Mechanisms for the continuation of the dialogue** that has been promoted by this workshop between researchers, regulators and industry should be considered. This may involve a similar or smaller group meeting every year or more frequently depending on the evolution of the disease.
- 3. Seek greater cooperation with organisations responsible for shipping, environmental management and quarantine. Biofouling has been identified as a possible route of introduction of OsHV-1 μVar as well as other diseases. Current attention appears to be focused solely on protection against pest species, but the risk of introduction of microorganisms needs to be considered. Measures to address the risk of consumers spreading the disease in oysters for consumption should also be considered.

DIAGNOSTICS

- 4. Support processes to **standardise diagnostic tests**, in particular PCR. Without limiting the active development of new and improved tests, researchers should support the current initiatives in the development of a chapter on OsHV-1 μ Var for the *OIE Manual of Diagnostic Tests for Aquatic Animals*. This chapter will provide guidance on diagnostic testing options and may have legal status for international trade if the disease is listed by OIE. Scientists working on the development and application of OsHV-1 μ Var diagnostic PCR techniques should ensure that they have input into the draft chapter when it is circulated to member countries for comment before it is considered for adoption (probably in May 2012).
- 5. Continue current efforts and exploit available opportunities to validate currently available tests where possible. If appropriate, utilise the results of large-scale surveillance (such as that currently being undertaken in Australia and UK) to better characterise the performance (diagnostic sensitivity and specificity) of key tests, using techniques that do not require a gold standard.
- 6. AAHL should consider preparation and distribution of **standard positive controls** for PCRs to assist in test standardisation and quality control. This must be done in response to requests from laboratories (Tasmania has agreed to pursue this issue).

RESEARCH

- 7. Undertake further **phylogenetic studies** to better understand the relationship between geographical isolates and thus possibly inform paths of spread of $OsHV-1 \mu Var$.
 - Continue work on **full genome sequencing** currently being undertaken at different laboratories, to assist better understanding of possible virulence and pathogenicity mechanisms.
 - Include sequences from Australian and New Zealand isolates (amongst others) in **phylogenetic analysis** being conducted in France, to determine the relationship between the viruses and possible paths of spread.
 - Investigate the use of other sequencing techniques to provide **higher resolution analysis** of molecular differences between isolates of OsHV-1 µVar.

- 8. Promote **collaboration between histologists** in France, Australia and elsewhere to more precisely characterise histopathology associated with OsHV-1 μVar infection.
- 9. Noting that France has developed an experimental infection model, establish a standardised **experimental infection model** to facilitate research into the effect of different risk factors. Several States and organisations have the capacity to undertake this work in Australia with SARDI, EMAI and USyd expressing interest to do so.
- 10. Undertake a range of specific studies to better understand **disease spread and transmission**.
 - Studies to investigate whether the virus is **transmitted vertically**, and, as has been suggested, whether methods are available to produce uninfected larvae from infected brood stock.
 - Studies into the **role of different species** (other than the Pacific oyster) in maintaining and spreading the infection. This should address the issues of (a) whether other species are able to become infected (with replicating virus) or merely become passive carriers (mechanical vectors of the virus), (b) the duration of infection or carriage, (c) their ability to spread the infection, and any possible role they may play in decreasing the viral load in the environment.
 - Studies into the **persistence of infection** including the existence of subclinical carriers.
- 11. Undertake research into the possibility of **inducing immunity** to viruses in oysters.
- 12. Continue the analysis of possible **entry pathways** currently being undertaken by DAFF.
- 13. Support epidemiological analysis of the role of different purported **risk factors** in causing the disease, with the aim of identifying possible control mechanisms or predicting periods of high risk for disease outbreaks.

SURVEILLANCE

14. Consider effectiveness of the **passive farmer reporting** system in preparation for the 2011-12 southern hemisphere summer risk period. The system should aim to provide rapid reporting of mortalities that may be associated with $OsHV-1 \mu Var$.

- Establish a simple unambiguous **definition of when reporting is required**. Current regulations generally include a mortality threshold (e.g. 5%) and the phrase 'unexplained mortalities'. Assessing mortality rates is often difficult.
- Consider ways to determine what constitutes **normal and abnormal mortalities**, and prioritise response and investigation on this basis.
- Clarify **disease reporting pathways** and responsibilities so that all stakeholders are aware of how, when and to whom to report, and what to do with any reports received.
- If possible, minimise any **negative consequences** of reporting. When a significant event, e.g. suspect exotic disease outbreak, is reported (and requiring immediate movement standstill), ensure that communication and mechanisms are in place to achieve rapid laboratory confirmation or exclusion, so the standstill does not cause unnecessary hardship to the industry.
- 15. With agreement between industry and regulators, and on a cost/benefit basis, consider development of a comprehensive system for reporting, managing and analysing data on **transfers of oysters** within and between estuaries, including movements of hatchery and wild spat. These data will provide a valuable resource for understanding the spread of OsHV-1 μ Var, for planning appropriate emergency control strategies to limit disease spread while minimising the impact on industry, and provide a basis for management of possible future diseases.
- 16. Consider development of systems to capture **data on risk factors** possibly associated with OsHV-1 μ Var infection and other oyster diseases. Data on risk factors can support epidemiological studies which aim to:
 - better evaluate the role of different risk factors
 - develop improved control measures
 - predict the risk of disease occurrence

Risk factors of interest relate to management and environmental factors as well as the host population at risk. To understand the role of possible risk factors, they must be measured in both affected and unaffected areas. Ideally, such a system would be in place before the southern hemisphere summer risk period.

Links should be established to existing data sources of interest, for example, databases of environmental data derived from data loggers.

- 17. Explore the possibility of establishing a **national industry-run**, **event-based database** to achieve the objectives of recommendations 144, 155 and 166. The database would capture data on:
 - Population distribution
 - Movements
 - Mortalities
 - Management and environmental risk factors

Any system should capitalise on existing activities such as the benchmarking project, and be designed to minimise the data collection burden on farmers (for example, using telephone or a digital diary for reporting) and minimise the data analysis and reporting burden (through automated analysis and alerts).

- 18. Establish the global distribution of OsHV-1 μ Var. There is little information available about mortalities or testing in the major oyster-producing countries of Asia (in particular) or other parts of the world. This should be an initiative of the OIE Reference Laboratory, through collaboration with scientists and regulators in the major oyster-producing countries.
- 19. Investigate any shellfish mortality to exclude OsHV-1 μ Var as a possible cause. Retrospective studies on previous scallop mortalities for OsHV-1 μ Var should be undertaken.

PREPAREDNESS

- 20. Further develop industry, state and national **contingency plans** to allow rapid response to mortality events that may be due to OsHV-1 μ Var. Components of the contingency plan should include:
 - Restricting movements of oysters, equipment and other shellfish out of affected estuaries
 - Establishing systems for rapid diagnostics (see recommendation 144)
 - Development of standard biosecurity guidelines for farmers and provide training in the application of the guidelines
 - Development of documented Good Management Practices for data collection, response, control and surveillance
 - Training of stakeholders in responsibilities and responses during an outbreak, including industry training in sample collection
 - Providing advice to industry on testing protocols and costs

- Providing advice to industry on management measures as well as technical, financial and personal resources available should the disease become established.
- 21. Develop effective communication with media outlets and the public, relating to disease outbreaks, that emphasises that the disease poses no threat to human health.

CONTROL

- 22. Continue and support research on selective breeding to develop oysters that are resistant to the virus.
- 23. In Australia, industry and regulators should support all measures to contain infection to the current two affected estuaries in NSW (Georges River and Parramatta River).
- 24. Consider potential management strategies to minimise the impact of the disease in affected areas:
 - Minimise the movement and handling of oysters to minimise stress during outbreaks or risk periods
 - Grow spat to a larger size before stocking after the end of the risk period, and attempt to grow them to marketable size before the start of the next risk period
 - Explore the use of alternative species that are not susceptible to the virus (for example, Sydney rock oysters (*Saccostrea glomerata*), flat oysters (*Ostrea angasi*) or pipis (*Paphies australis*)), either as replacement product, or to decrease the viral load in the water column and limit the impact on Pacific oysters (*Crassostrea gigas*).

3. INTRODUCTION

OBJECTIVES

The objectives of the workshop were to:

- Review current knowledge about OsHV-1 µVar, in particular in relation to its detection, epidemiology and current global distribution
- Assess current practices for surveillance, prevention and control of the associated disease and provide recommendations to national, regional and global authorities for their improvement

• Identify priorities for further research, and plan and coordinate research activities between international partners

VENUE

The workshop took place at the Pullman Reef Hotel, 35-41 Wharf Street, Cairns, Queensland, Australia which was the same venue as the First Australasian Scientific Conference on Aquatic Animal Health held during the preceding week. The program commenced at 9:00 am on Saturday 9 July and ran through to 5:00 pm on Sunday 10 July.

PARTICIPANTS

See appendix.

PROGRAM

See appendix.

FORMAT

The workshop did not consist of formal presentations by different participants, but instead focused on structured discussion of a series of topics and brief informal presentations from those with knowledge and experience of the topic.

Participants were invited to contribute to the discussion for those topics in which they had experience or expertise. Participants were requested to prepare brief materials to assist the discussion and documentation, either as a MS Word document or a few MS PowerPoint slides.

A great deal of material was covered and a relatively large number of participants (over 30) attended. The facilitators therefore limited discussion on some topics in the interest of time. It was thought likely that a disproportionate amount of time would be given for contributions from those participants with the most experience of the disease, but questions and interactions were received from all participants.

FACILITATORS

The workshop was facilitated by Drs Angus Cameron (Director, AusVet Animal Health Services) and Mark Crane (FRDC Aquatic Animal Health Subprogram Leader and Research Team Leader, AAHL Fish Diseases Laboratory, CSIRO Livestock Industries).

4. THE DISEASE

WHAT IS THE DISEASE? OSHV-1 μ VAR

The workshop focussed its interest on the detection and identification of the Ostreid herpesvirus-1 microvariant (OsHV-1 μ Var) in the presence of high mortalities amongst Pacific oyster (*C. gigas*) populations, whether farmed or wild.

The disease associated with infection by OsHV-1 μ Var has been given several names, for example, in New Zealand it was initially referred to as a herpes virus but after consideration of the possible negative effect this might have on market perceptions it was changed to Juvenile Oyster Mortality Syndrome (JOMS). First reports from NZ suggested that some supermarket chains ceased oyster purchases, but more recent information noted that the initial public reaction was motivated by an initial "giggle factor" association with human Herpes Simplex but that little lasting impact has been seen in the marketplace. In New South Wales, the first outbreak occurred just prior to the Christmas market season and it was therefore decided to refer to the disease as Pacific Oyster Mortality Syndrome (POMS) in an attempt to reduce any negative impact to the industry.

For the purpose of the workshop it was agreed that the disease under discussion was high mortality in Pacific oysters associated with the presence of OsHV-1 μ Var. This excludes "summer mortality" not associated with OsHV-1 μ Var, and disease caused by strains of OsHV-1 other than OsHV-1 μ Var.

5. EMERGENCE AND DISTRIBUTION

WHERE IS IT?

Infection with "classical herpes" viruses is known to occur in a large number of mollusc species and is found widely, however OsHV-1 μ Var has thus far been declared in the EU, Australia and New Zealand.

EMERGENCE AND DISTRIBUTION IN EUROPE

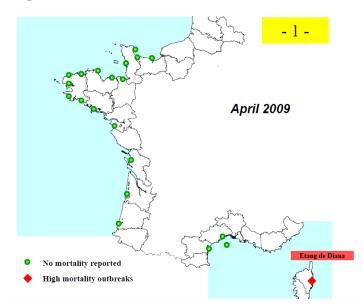
France

France reported high mortalities in Pacific oysters (*C. gigas*) associated with Oyster Herpes Virus in the presence of *Vibrio splendidus* in April-May 2008¹ during

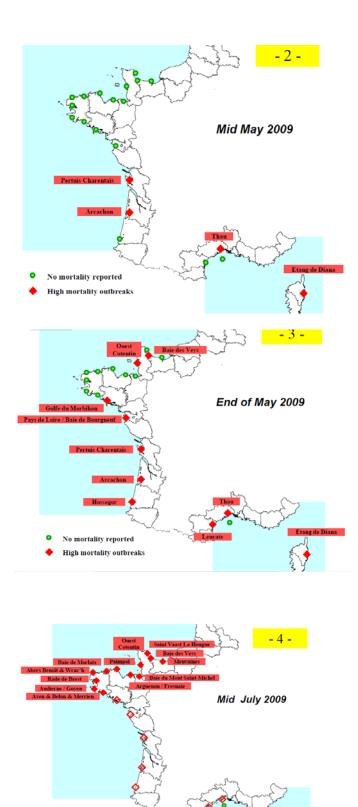
¹ <u>http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=7288</u>

investigations into widespread mortalities along the French coasts. Mortality rates in spat (up to 12 months old) and in juveniles (up to 18 months old) between 40% and 100% were reported. The pattern of occurrence was not a smooth progression from south to north as has been observed in outbreaks of classical OsHV-1, but rather outbreaks occurred at scattered sites along the coastline prompting the hypothesis that the disease was spread by the movement of animals.

In 2009² the disease progressed more smoothly from the south to the north as water temperatures increased. This suggested that the virus may have already been widely distributed by this stage and outbreaks were initiated by an increase in temperature.



² <u>http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=8265</u>

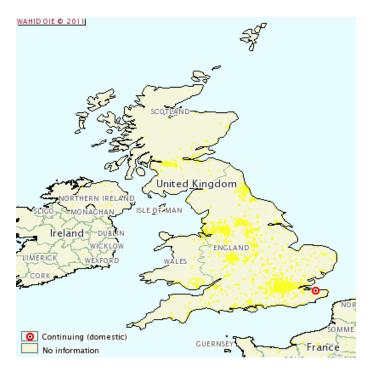


No mortality reported High mortality outbreaks

Decrease or stop of mortality outbreaks
 Mortality outbreaks finished

Ś

UK experienced high mortalities in 2008 and 2009. In July 2010³, the UK reported significant mortalities associated with OsHV-1 μ Var in <u>Whitstable Bay</u> in the Thames estuary and in Grouville Bay, South-East Jersey⁴. The UK has managed to contain the virus to the outbreak sites after immediate containment measures were established at the time of detection.



UK

³ <u>http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=9527</u>

⁴ <u>http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=9571</u>



Republic of Ireland

The presence of OsHV-1 μ Var was confirmed in 2009⁵ in samples from farms experiencing high mortalities from three bays in the Republic of Ireland (RoI). Approximately 16 bays were affected the following year. All sites found positive for OsHV-1 μ Var had been stocked with spat from France. Sites which had not received shellfish from France all tested negative for OsHV-1 μ Var. The RoI has since been able to declare a number of areas disease free but the disease remains widespread.

⁵ <u>http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=8503</u>



Other European countries

Spain, the Netherlands and Italy⁶ have detected OsHV-1 μ Var but have experienced no mortalities associated with the disease.

Most recently, in June 28 2011⁷, the Netherlands reported high mortalities in the presence of OsHV-1 μ Var.

EMERGENCE AND DISTRIBUTION IN AUSTRALIA AND NEW ZEALAND

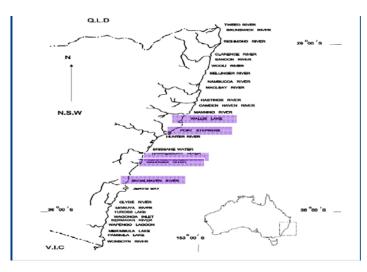
Australia

The Sydney rock oyster (*Saccostrea glomerata*) is the predominant oyster species farmed in New South Wales (NSW). There are presently 5 estuaries in NSW where triploid Pacific oysters are farmed (Wallis Lake, Port Stephens, Hawkesbury River, Georges River and Shoalhaven River). Diploid Pacific oysters are also farmed in Port Stephens. Excellent maps of these farmed areas and details of lease holdings

⁶ Detection of OsHV-1 µvar and Bonamia exitiosain farmed oysters in Italy during 2010. <u>http://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/presentations/ostreid_he</u> <u>rpesvirus_bonamia_exitiosa_italy.pdf</u>

^{7 &}lt;u>http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=10749</u>

exist. There are also wild populations of Pacific oysters in estuaries south of Hastings River, however the full extent of these populations is much less well understood.

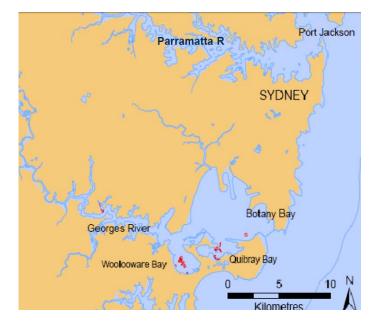


Reports of high mortalities in 4mm–15cm farmed spat and wild Pacific oysters were received from several farmers in Woolooware Bay, Georges River estuary in late November 2010. Poor water quality nearby had also been reported just prior to the reports of mortalities. The mortalities were investigated and a response under the NSW Fish Kill Protocol was initiated. Water quality samples showed no significant contamination. Initial histopathology was inconclusive. OsHV-1 μ Var was confirmed in December 2010 at AAHL and an immediate notification was made to OIE. Movements of stock and farming infrastructure were controlled between estuaries but not within the estuary. Sales of healthy oysters from affected areas for human consumption were permitted.

During population distribution surveys in preparation for testing wild oysters in Sydney Harbour, mortalities of wild Pacific oysters were observed in the Parramatta River in January 2011 and OsHV-1 μ Var was identified⁸. Anecdotal reports from the public suggested that the mortalities may have started four months earlier.

Sites in the upper reaches of the Georges River showed mortalities in wild Pacific oysters in early 2011 but cohabiting populations of Sydney rock oysters appeared unaffected. Previous mortalities in wild populations would probably not have been reported therefore it is impossible to say that Woolooware Bay was the first site where the disease occurred.

⁸ <u>http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=10136</u>



The oyster growing industry in Australia, but particularly in NSW, has a heightened state of awareness. Unexplained mortalities in Pacific oysters in other NSW estuaries during the first six months of 2011 were reported, samples submitted and OsHV-1 μ Var was not identified. This provides good evidence that the disease is not present in farmed Pacific oysters elsewhere.

New Zealand

New Zealand investigated mortalities on a farm in the Coramandel Peninsula, North Island in March 2010. Other reports of high mortalities were received from around Auckland and the Bay of Islands. An environmental problem was initially suspected. Samples were sent to a private laboratory – MAF was not informed. Deaths stopped after 7 days, winter came, and notifiable diseases were ruled out by the private laboratory.

In late November 2010⁹, simultaneous mortalities were reported in the same areas and had been preceded by a rapid rise in water temperatures (3°C in one week). MAF was informed, investigated and determined that OsHV-1 was associated with the mortalities. Sequence analysis indicated that the isolates shared a 13bp deletion in the non-coding area of the C-region, although other minor point mutations in the coding area of the C-region were not present in the NZ isolate as compared with the European isolate of OsHV-1 μ Var. Areas affected in March appeared less affected in

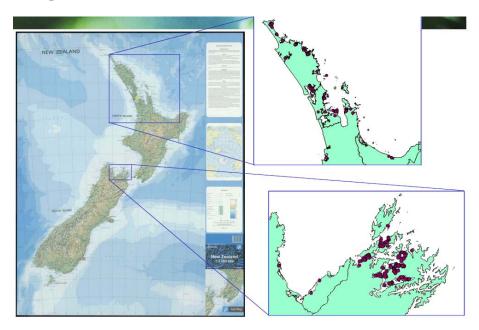
⁹ <u>http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=10013</u>

November. Mortalities continued to mid-December. There are two areas in the North Island where there have been no introductions through infected spat or animal movement and these have remained OsHV-1 μ Var free. No mortalities were reported in the South Island.

New Zealand uses approximately 90% wild spat in farming. Most spat are caught on sticks in harbours on the west coast of the North Island and are moved to other areas. Half a million sticks are taken into the harbours to capture spat and then taken away and returned the next year. These sticks are often not cleaned.

Wild spat showed 50% mortality. Larger oysters seemed to be greatly affected. Hatchery spat experienced very high mortality, up to 100%, and it was speculated that this was due to their higher growth rate. Spat were seen to die within 48 hours of being put out to sea, although a period of 6 days is more likely.

A hatchery in the South Island, which had larval crashes, detected the presence of OsHV-1 μ Var. Movements of oysters between the North and South Islands are infrequent. Brood stock however was moved from North to South.



EVIDENCE FROM OTHER PARTS OF THE WORLD

Recent high mortalities in the US are not known to be associated with OsHV-1 μ Var at this stage. There is some question regarding the PCR test the US has used during investigations of high mortalities and whether they would have detected OsHV-1 μ Var had it been present.

China, Japan and Korea are major producers of Pacific oysters. Investigations involving oyster samples from these countries allowed detection of oyster herpes virus, OsHV-1, but not the new μ Var. Recent large scale mortalities from these countries have not been reported. The genetic similarity between OsHV-1 μ Var and east Asian herpes isolates (see phylogenetic tree on page 30) raises the question of whether the disease could be present in this region despite the absence of reports.

6. IMPACT

Estimating impact is difficult and complex and can include sociological, economic, regulatory and institutional factors amongst others.

	FRANCE	UK AND EUROPE	AUSTRALIA	NEW ZEALAND	REST OF THE WORLD
First outbreak	April 2008	UK: July 2009 IRE: 2009 NL: June 2011	Nov 2010	March 2010	None reported
Growing areas affected	100%	66% Ireland 3% England	20% in NSW* 1% nationally by lease area	73%	
Spat mortality <12mths	High	High	Highest	80-100%	
Juvenile mortality 12–18mths	Medium	Medium	Higher	25-42%	
Adult mortality >18 mths	Low	Low	High	8-20%	
Is there a decrease in the impact of disease over time?	No (same)	No (same)	Not applicable	Exposed populations less affected than naïve	
Economic Impact to date	Not apparent but little available data		10% estimated loss for NSW Pacific oyster production	Farm production fell by 25%	
Potential future impact			SA and Tas: Very high due to SA reliance on one hatchery in Tasmania and plan to expand export markets	Current 30% export to AUS, 30% to SEA, 30% to Pacific. Listing of disease may affect exports	

SUMMARY OF IMPACT IN DIFFERENT LOCATIONS

*Refers only to the 5 estuaries currently used for Pacific oyster culture

France

Significant widespread mortalities have been reported since 2008. The economic losses caused by the disease have not been estimated because of a lack of data. Subsidies and compensation schemes may lessen the impact to producers. The slow growth of Pacific oysters and lower mortality in adults in Europe may mean that the impact on marketable product is yet to be seen. French producers are reported to be increasing the number of spat stocked to compensate for expected mortalities. This strategy may also be masking the effect of disease on total production.

UK

The disease has been successfully contained to one small bay in the Thames estuary and in Jersey thus far, so the economic impact to the oyster growing industry has been limited.

The Republic of Ireland

Widespread disease has been detected (16 bays) however some areas have been able to be declared free from the disease, permitting movement and trade from and within these areas.

Other countries in Europe

Impacts in other European countries are thought to be minimal given the lack of mortalities attributable to the disease. The impact in the Netherlands following a recent report of mortalities is as yet unknown.

Australia

The triploid Pacific oyster industry in NSW was worth an estimated AUD \$4.5 million in 2009/10 financial year. Significant economic losses were experienced by affected farms in Georges River. There is a potential high future impact in Australia because of the reliance on hatchery (triploid and diploid) spat. According to the New Zealand experience, mortalities in hatchery spat are higher than in wild spat, although this difference has not been demonstrated in France. If the French strategy of increasing stocking of spat was adopted in Australia the costs for production would increase due to the cost of the extra hatchery spat.

There is an unknown potential impact on Sydney rock oysters, although no mortalities have been reported (despite their close proximity to affected Pacific oysters).

Pacific oysters are not native to Australia and in some areas they are considered a pest. This is the reason for the requirement in NSW to only farm triploids in all estuaries except in Port Stephens.

New Zealand

A lower impact has been perceived in New Zealand because the industry is mostly reliant on wild spat which have experienced lower mortalities. Nevertheless production has been reduced by between 25–33%. New Zealand exports oysters to Australia (30%), Asia (30%), the United States, Japan and the Pacific Islands.

Lower overall water temperatures are also seen as an advantage as this may reduce the likelihood or extent of disease outbreaks. Overstocking is seen as a more viable strategy due to the use of wild spat.

Other countries

The major producers of Pacific oysters are China, Korea, Japan, France, US, Canada and South America (in order of importance). The potential impact of OsHV-1 μ Var on production in these countries is enormous, and their collaboration could be sought for test development or surveillance activities.

ZOONOTIC POTENTIAL

Herpes viruses are normally highly host specific. Despite the widespread presence of Oyster Herpes Virus and OsHV-1 μ Var in France there is no evidence of human infection or impact. It is considered to pose no threat to human health.

7. CHARACTERISATION

DEFINITION OF OSHV-1 µVAR

During the initial outbreaks in France, OsHV-1 was detected using conventional PCR in 2008, and qPCR in 2009 and 2010. The isolates were sequenced and comparisons made with the OsHV-1 reference strain (GenBank # AY509253^{10 11}) using open reading frames 4 and 43 (ORF4 and ORF43). The C region (corresponding to the internal and terminal long repeats and which includes ORF4)

¹⁰ <u>http://www.ncbi.nlm.nih.gov/nucleotide/48696722</u>?

¹¹ http://www.ncbi.nlm.nih.gov/nucleotide/41352386?

is widely used to diagnose classical herpes virus (OsHV-1) and this is why it was first targeted for study. A number of differences were systematically detected:

- In the microsatellite zone of the non-coding part of ORF4, there is a 13 base pair deletion;
- There are two mutations in the coding region of ORF4;
- A number of other differences in the non-coding zone of ORF4; and
- Differences in the IA1-IA2 fragment of ORF43

More detailed genetic analysis was then conducted in France using up to 8 different ORFs. ORF4, ORF37 and ORF43 were identified for more detailed analysis using 79 French isolates, which showed that there was a further systematic 604 base pair deletion corresponding to the total lack of 2 ORFs (36 and 37) and a partial lack of ORF38. The deleted genes code for membrane proteins.

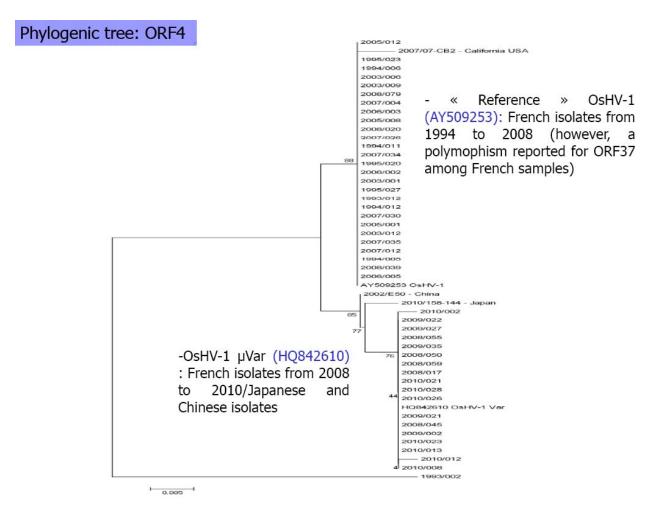
On the basis of these changes, the new strain was named OsHV-1 microvariant (μ Var). While changes may appear in various areas of the genome, it was agreed that the 13 base pair deletion in the microsatellite zone of the non-coding region of ORF4 should be used to define OsHV-1 μ Var for diagnostic purposes. This definition is consistent with that proposed in the EFSA opinion¹²

"According to the Council Regulation 175/2010/EC, OsHV-1 µvar means a genotype of the virus Ostreid herpesvirus-1 (OsHV-1) which is defined on the basis of partial sequence data exhibiting a systematic deletion of 12 base pairs in a microsatellite zone of the ORF 4 in comparison with OsHV-1 (GenBank # AY509253). OsHV-1 µvar was later defined by Segarra et al (2010) on the basis of numerous mutations in comparison with the sequence of the reference virus (Davison et al; , 2005) in two different ORFs, the C region (ORF4) and the IA region (ORF43). The number of deletions in ORF4 is contentious. Council Regulation 175/2010/EC states there are 12 deletions, which is also stated in the text of the Segarra et al., (2010) publication, although Fig 4 of the same publication shows 13 deletions."

The ORF4 of 52 isolates was used to generate a phylogenetic tree. Analysis included classical OsHV-1 isolates from France from between 1993 and 2008, isolates of OsHV-1 μ Var from France between 2008 and 2010, and further isolates of oyster

¹² Scientific Opinion on the increased mortality events in Pacific oysters, *Crassostrea gigas*, EFSA Journal 2010;8(11):1894 <u>http://www.efsa.europa.eu/en/scdocs/doc/1894.pdf</u>

herpes viruses from USA, Japan and China. The result of the analysis is shown below. Within ORF4, there is clear differentiation between the classical OsHV-1 and OsHV-1 μ Var isolates identified in France, but virtually no differentiation between isolates within these two groups. The US strain groups closely with the classical OsHV-1, while the Chinese (2002) and Japanese (2010) strains are much more closely related to the French OsHV-1 μ Var isolates.



No sequence data is yet available from Australia for comparison with the French OsHV-1 μ Var or classical OsHV-1 isolates, although this work is currently underway. In New Zealand, at least 3 isolates from separate areas have been sequenced and compared to both the OsHV-1 reference strain and the published OsHV-1 μ Var sequence, for ORF4. They were all found to be almost identical (two bp differences and other minor point mutations in the coding area of the C-region were not present in the NZ isolate as compared with the European isolate of OsHV-1 μ Var) to OsHV-1 μ Var in the C2/C6 region.

		1	10	20	30	40	50	60
NZ-81 (22:06	I ATAGATGT(I BATGTGCGGCA	I AAGATGAATG	GCAAGATACA	I CAATGAGCTAT	T PT <mark>A</mark> CCCGACC <i>I</i>	AC
OsHV-1 (C2-C6	ATAGATGT	GATGTGCGGC	AAGATGAATG	GCAAGATACA	CAATGAGCTAI	fT <mark>g</mark> cccgacc <i>i</i>	AC
μVar (C2:C6	ATAGATGT	SATGTGCGGC2	AAGATGAATG	GCAAGATACA	CAATGAGCTAI	TT <mark>A</mark> CCCGACC	4C
NZ-81 0						GGTTCCACAA		
OsHV-1 (GGTTCCACAA		
<u>µVar</u> (C2:C6	AAACCTAAG	CGTTGTATTCO	GATTACGGAT	TAAGAAAATG	GGTTCCACAAI	СТААААТТА	4A
NZ-81 (C2:C6					<mark>a</mark> aaaagtat <i>i</i>		
OsHV-1 (<mark>-</mark> AAAAAGTAT <i>i</i>		
<u>µVar</u> (C2:C6	-AA <mark>C</mark> CCAC	ATGGGGGCCA	AGGAATTTAA	A <mark>G</mark> CCCCGGGG	<mark>A</mark> AAAAAGTAT <i>I</i>	1AATAGGCGC(ЗA
NZ-81 (AGTATACCAC		
OsHV-1 (AGTATACCAC		
<u>µVar</u> (C2:C6	TTTGTCAG				AGTATACCACA	ACTGCTAAAI	ſΤ
				satellite	V/////////////////////////////////////			
NZ-81 0						AAATGCAGCCI		
OsHV-1 (AAATGCAGCCI		
<u>µVar</u> (C2:C6	AACAGCAT	CTACTACTAC:	FACT <mark>G</mark>	AA	AAATGCAGCCI	TTCACAGAAI	ſΤ
NZ-81 (ACTTTTTCAT		
OsHV-1 (ACTTTTTCAT		
<u>μVar</u> C	C2:C6	TTGCACCTI	GACCAAAGC	CATCACATCA	GCCAGCAACG	ACTTTTTCAT	CAACCAGACGA	4G
NZ-81 (C2:C6	GTTAACAT	GCGACATTTG'	TAAAGAGCTC	GTCTCTTTC <mark>G</mark>	ATTGC <mark>G</mark> AAGA1	FAAAGTCGTG	GC
OsHV-1 (C2-C6	GTTAACAT	GCGACATTTG	FAAAGAGCTC	GTCTCTTTC <mark>G</mark>	attgc <mark>g</mark> aagai	FAAAGTCGTG	GC
<u>µVar</u> (C2:C6	GTTAACAT	GCGACATTTG	FAAAGAGCTC	GTCTCTTTC <mark>A</mark>	attgc <mark>a</mark> aagai	TAAAGTCGTG	ЗC
NZ-81 (C2:C6	ATCATTGG	CTGCAGTCAG	ATCTGACATA	.CCCATAGAAG'	TCACGGAACG	CAAAGACCTGA	AA
OsHV-1 (C2-C6	ATCATTGG	CTGCAGTCAG	ATCTGACATA	.CCCATAGAAG'	TCACGGAACG	CAAAGACCTGA	AA
µVar C	C2:C6	ATCATTGGG	CTGCAGTCAG	ATCTGACATA	CCCATAGAAG'	ICACGGAACGO	CAAAGACCTGA	AA

It was agreed that genetic studies were valuable to understand both the origin and possible mode of spread of the virus, as well as understanding the mechanisms of pathogenicity. Inclusion of both Australian and New Zealand isolates, as well as those from other European countries in the phylogenetic analysis was seen as a priority, requiring collaboration between the different countries.

Full genome sequencing has not yet been completed although it is anticipated that a sequence will soon be available. This should provide insight into the differences between classical OsHV-1 and OsHV-1 μ Var that may assist explain the apparent increase in virulence.

Using ORF4 failed to provide adequately fine resolution to distinguish between the French isolates or the New Zealand and the French strain. In order to understand the relationships between these viruses, it was suggested that more or different sequences need to be examined.

VIRAL POPULATION SHIFTS

OsHV-1 μ Var was first identified in France in 2008. In that year, 42% of herpesvirus isolates identified during mortality events were OsHV-1 μ Var while the remainder were classical OsHV-1. In 2009 and 2010, 100% of herpesvirus isolates detected during mortality events were OsHV-1 μ Var. This suggests that the new strain had successfully replaced the classical strain as a cause of morbidity in oysters within the space of one year.

The implications of this observation in France are that OsHV-1 μ Var may be able to compete well with the classical strain, and that it appears to have been rapidly disseminated throughout virtually the entire French Pacific oyster population.

STABILITY OF THE VIRUS

The transcription mechanism in the replication of double-stranded DNA viruses, such as Herpes viruses, has built-in quality checks, significantly reducing the errorrate compared to RNA viruses such as influenza. Herpes viruses are therefore relatively genetically stable.

The stability of OsHV-1 viruses is nevertheless uncertain, as little work has been done in this area. It was noted that in cases where there is heavy infection in large populations (as may be the case in intensive oyster growing areas), there is greater opportunity for mutation because of the rapid replication and large number of viral generations possible.

8. DIAGNOSTICS

A number of diagnostic tools are available for detecting suspect cases of OsHV-1 infection, including histology and *in situ* hybridisation. Molecular techniques (PCR) are used for confirmatory diagnosis of infection with OsHV-1 μ Var, and surveillance for subclinical infections.

CLINICAL SIGNS

Affected oysters are usually found dead with decomposing material or empty shells. New Zealand reports that early cases show gaping and weak closure reflex. No characteristic clinical or gross pathological changes have been noted.

HISTOLOGY

Histology is an important tool in the diagnosis of OsHV-1 μ Var, primarily due to its use in excluding other possible causes of mortality, including bacterial and parasitic diseases.

Histology in oysters affected with OsHV-1 μ Var indicates non-specific histopathology (inflammation), and to date, no characteristic histopathological lesions have been identified. Experiments are underway in Australia involving the serial sampling of naïve oysters introduced into an environment that was previously habitat for infected oysters, and histologists indicate that they are beginning to get a picture of the progression of the infection that may be useful for diagnosis. Histologists in France also indicate that they are able to identify pathological changes associated with OsHV-1 infection. It was agreed that these new results offer significant promise and that collaboration between histologists in affected countries should be encouraged. It was suggested that web-based tools developed by ABIN could represent a useful resource for histopathologists to collaborate using real-time discussion and shared viewing of high quality histopathology images.

In the past, inclusion bodies have been often recognised as a feature of herpes virus infections. In Australia, histologists have noted that inclusion bodies have not been identified in OsHV-1 μ Var samples. In France, inclusion bodies have not been seen with either classical OsHV-1 or OsHV-1 μ Var infections in *C. gigas*.

ELECTRON MICROSCOPY

Electron microscopy has been used in France and is underway at EMAI in Australia. There is interest in the possibility of using electron microscopy to understand the way in which the virus may acquire its coat and make its way through the cell membrane, given that genetic deletions have occurred in areas that code for membrane proteins. To date, in France, no differences have been noted between classical OsHV-1 and OsHV-1 μ Var by electron microscopy.

POLYMERASE CHAIN REACTION (PCR) TESTS

Polymerase chain reaction (PCR) tests are used to identify specific genetic sequences in samples. PCR tests are very sensitive because very small amounts of genetic material are repeatedly replicated to levels that allow detection. The sequence to be replicated is determined by primers based on specific nucleic acid sequences that are designed to match only a small part of the target organism's genome, making the tests very specific.

If sufficient genetic material matching the primers is present in the sample, a PCR test will give a positive result. This doesn't necessarily mean that the oyster was infected, or that the virus was causing the disease. Passive carriage of virus, or the presence of non-infectious fragments of viral DNA may both result in a positive test result. The extreme sensitivity of the test means that cross contamination due to poor sampling technique and/or poor laboratory technique can also cause false positive results.

In order to provide a positive reaction, the primer must exactly match a small portion of the virus' DNA (n.b. for some PCR tests a small degree of mismatch may be tolerable but is likely to reduce the test's sensitivity and specificity). If a mutation has occurred in just a single base pair in the region targeted by the primer, then the test may be negative, even though the rest of the viral sequence may be identical.

A number of different PCR tests for OsHV-1 and OsHV-1 μ Var have been developed and are being used in different laboratories. These different tests are distinguished either by the primers they are using or the technology used to implement the test (conventional PCR, real-time PCR, TaqMan PCR).

Currently PCR tests used to identify OsHV-1 include real-time PCR using SYBR green and a TaqMan formats. These tests are not able to differentiate classical OsHV-1 from OsHV-1 μ Var.

Characterisation of OsHV-1 μ Var is currently being done using conventional or nested PCR using primers targeting the C2/C6 segment (the location of the 13 base pair deletion). A number of laboratories have developed different primers targeting this segment. A TaqMan real-time assay to directly identify OsHV-1 μ Var is currently being developed in France to support EU surveillance requirements.

The development of multiple new and subtly different PCR tests in response to the recognition of a new disease-causing agent is not surprising, and provides an opportunity for improved tests to be developed. However, this process results in a lack of standardisation (different tests may give different results on the same sample), potentially undermining confidence in test results. In addition, the process of determining which test is appropriate in a particular situation normally involves comparison of candidate tests to an accepted 'standard' test with known performance. In the case of OsHV-1 μ Var, all tests are new and none have yet been

adequately validated to provide reliable information about their performance. This issue is discussed in the next section.

A summary of different published PCR tests is provided in Appendix B of the EFSA report¹³. In addition to published methods, some laboratories (including EMAI) are using, as yet, unpublished modifications in an attempt to improve the tests' performance.

Tristan Renault from IFREMER is currently drafting a chapter on diagnostic tests for OsHV-1 μ Var for the OIE Manual of Diagnostic Tests for Aquatic Animals. This is seen as an important step in achieving improved test standardisation. OIE member states will have an opportunity to comment on the Manual chapter before its expected adoption in May 2012. The OIE Manual normally only contains information on tests for listed diseases (those reportable to the OIE), so the inclusion of this chapter is seen as an unusual step.

IN SITU HYBRIDISATION

This test uses a labelled nucleic acid probe to bind to specific genetic material in histological slides. RNA probes are used to bind to messenger RNA produced by the virus during replication. This technique can distinguish between virus that is being passively carried by the oyster and virus that is actively replicating in the tissues. Furthermore, it allows visualisation of the location of the virus in specific tissues, identifying the preferred sites of infection and replication.

This approach is being used to study OsHV-1 μ Var in a longitudinal study in New Zealand. Currently, it is being used primarily as a research tool rather than a diagnostic tool.

PROTEOMICS

Proteomics involves testing for protein biomarkers (products of infection) that may be switched on before PCR testing is able to reliably detect the infection. This option was raised as a possibility, but no work has been undertaken in this area.

RAPID FIELD TEST

Effective prevention of spread of the disease from new outbreaks is likely to depend on rapid recognition, rapid reporting and introduction of a movement standstill

¹³ Scientific Opinion on the increased mortality events in Pacific oysters, *Crassostrea gigas*, EFSA Journal (2010), 8(11):1894, 54-55.

until diagnostic tests are able to determine whether or not the mortalities are caused by OsHV-1 μ Var. Controls on the movement of oysters out of an estuary while waiting for test results are likely to cause hardship to producers, and if most cases prove not to be due to a new disease requiring movement restrictions, compliance and reporting rates are likely to drop rapidly. This issue emphasised the need for rapid diagnosis to minimise the negative impact of temporary movement restrictions on producers.

The potential value of developing a rapid field test was raised and discussed. It was agreed that laboratory tests are more reliable than is possible with rapid field tests (especially when results are negative) and that the speed with which laboratory tests can be conducted was adequate in most situations. However, it is important for laboratories and disease control authorities to know when rapid field testing is important and when it isn't. For routine testing, or surveillance work, rapid results are not critical. However, whenever a new incursion of OsHV-1 μ Var into a previously uninfected area is suspected (and therefore results in a movement ban), laboratories should be clearly informed that urgent test results are required.

The conclusion was that there is no real need for the development of a rapid field test (considering the time and cost that would be involved in such development) and that current laboratory tests are capable of providing adequate test turnaround if communication and transport systems work as they should.

Editor's note: Post workshop, information on a LAMP assay (Ren et al., 2010) which has potential as a rapid field test was provided.

Currently, OsHV-1 μ Var PCR testing is only conducted in Australia at EMAI (NSW) and AAHL (in Geelong), and in EU. Both South Australia and Tasmania expressed interest in implementing the PCR in their own state laboratories to further speed exclusion testing. Under agreed arrangements in Australia, testing for suspect exotic disease outbreaks is conducted at AAHL. If OsHV-1 μ Var was declared to be an endemic disease in Australia, state laboratories would be expected to establish testing capability.

TEST VALIDATION AND STANDARDISATION

Some aspects of validation and standardisation include:

• Ensuring that when one laboratory identifies a sample as positive for OsHV-1 μ Var, another laboratory would provide the same conclusion for the same sample. This requires that:

- $\circ~$ Laboratories are using the same molecular definition of OsHV-1 $\mu Var,$ which is normally determined by PCR configuration and primers used.
- Ring testing has ensured that the laboratories are meeting the same performance standards
- Understanding the performance and use of the test, including the following aspects:
 - o Fitness for intended purpose(s)
 - o Optimisation
 - o Standardisation
 - o Robustness
 - o Repeatability
 - o Analytical sensitivity
 - o Analytical specificity
 - o Thresholds (positive and negative cut-offs)
 - o Diagnostic sensitivity
 - o Diagnostic specificity
 - o Reproducibility
 - o Ruggedness

Currently, there is no standardisation in the use of tests for OsHV-1 μ Var, nor are any of the tests in use fully validated according to the OIE guidelines for test validation¹⁴. While this situation may appear to pose significant problems, it must be considered in context:

- o Standardisation
 - The OIE Manual chapter on OsHV-1 tests is in preparation. This should provide guidance on recognised tests. If, in the future, OIE lists OsHV-1 µVar, then use of the tests described in the Manual would be a legal requirement for international trade purposes.
 - Most tests are targeting one of two regions, using similar primers. It would therefore be expected that they should give very similar results.
 - For the current national survey in Australia, for which EMAI and AAHL are doing the testing using different tests as part of the same surveillance program, procedures are in place to ensure that results will be consistent. This includes:
 - A comparison of both tests that has been undertaken at AAHL and indicated that the results are consistent, and

¹⁴ <u>http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2010/1.1.2_VALID.pdf</u>

- Follow-up testing of any positive samples detected at either laboratory with a second confirmatory test at the other laboratory.
- IFREMER conducts inter-laboratory ring tests in Europe. Similar systems are in place in Australia and New Zealand and could be applied to the OsHV-1 PCR.
- Standard positive control samples are required for test quality assurance. AAHL has undertaken to provide these samples for Australian laboratories but progress is slow. Australian laboratories should continue to exert pressure to ensure that this is done.
- o Validation
 - In reality, very few diagnostic tests in current use for any disease are fully validated to meet the OIE standards. Most of the current tests have been evaluated in the laboratory in which they were developed, including an assessment of robustness, repeatability, analytical sensitivity and analytical specificity. Issues of fitness for purpose, diagnostic sensitivity and specificity, and choice of suitable thresholds are often more complex and less well examined.
 - By their nature, it can be confidently expected that the diagnostic sensitivity and specificity of the PCR tests are likely to be very good (in terms of detecting the presence of viral genetic material, rather than as an indicator of active infection).

The current Australian survey will provide valuable information to help characterise the diagnostic specificity of the tests being used. If no virus is detected outside the currently identified estuaries (Georges River and Parramatta River), then it can be assumed that all other samples are from non-infected populations and allow estimation of diagnostic specificity. On the other hand, a small number of positive test results would complicate the matter.

Statistical methods now exist which enable the diagnostic sensitivity and specificity of tests to be estimated, even when a 'gold standard' test, providing the 'true' disease status of animals is not available. These methods require the use of two different and unrelated tests in at least two populations (in which the disease is present at different levels). Surveys and testing in different parts of the world (for instance New Zealand, Australia and France) may provide an opportunity for collaboration to field validate a number of tests.

Fitness for purpose is an important consideration in test validation. Different tests and test combinations will be needed (and possible different cut-offs or thresholds for the same test) for the following situations:

- Diagnosis of the disease as the first occurrence in a previously unaffected area
- Diagnosis of the disease in an area in which it is considered to be endemic
- Surveillance to demonstrate freedom from the virus
- Surveillance to measure the prevalence of infection in an endemic area
- Studies to assess host susceptibility (requiring differentiation of infection from passive carriage of the virus)

9. INTERNATIONAL RESPONSE AND REGULATION

AUSTRALIAN AND NEW ZEALAND REGULATORY RESPONSES

Australia

Following the report of mortalities in Woolooware Bay in November 2010 voluntary movement controls were instantaneous and industry cooperation was good. Sales of product to the marketplace for human consumption were permitted. Reporting of mortalities and movements is a long-standing mandatory requirement of oyster farmers in NSW, industry was widely reminded of its importance as soon as the virus was identified, and free testing was provided to the industry.

In general, official quarantine restrictions may not be put in place immediately, for example, if unexplained mortalities are not thought to be infectious disease related. NSW authorities may require confirmation of the disease prior to instigating controls, and diagnosis can take several days. NSW has all oyster leases mapped and in a GIS layer.

South Australia and Tasmania are able to put movement controls in place in the event of a suspected (but not necessarily identified) disease at the discretion of the State Chief Veterinary Officer, however a report of unusual mortalities would not necessarily in itself justify immediate movement restrictions. Fatigue from within the industry in the event of frequent shutdowns due to "unexplained mortalities" would be a likely consequence – and could lead to a reduction in reporting. Movement controls can be difficult to enforce. In certain circumstances when movement restrictions are in place, it might be considered safe for healthy product from affected areas to be sold for human consumption.

Early reporting and diagnosis is important to ensure a rapid response to disease outbreaks which may limit disease spread and increase the opportunities for eradication. The importance of removing disincentives, and increasing incentives, for farmers to report suspect disease or unusual mortalities was recognised. Given the detection of OsHV-1 μ Var in NSW, in the event of large mortalities, particularly in Pacific oysters elsewhere in Australia, it would be logical to assume that OsHV-1 μ Var might be the cause. Under these circumstances immediate movement restrictions and testing to confirm or exclude OsHV-1 μ Var would be appropriate.

The turnaround time for diagnostic results, if slow, could result in unnecessarily long restrictions, impacts on business, and lead to a reduction in reporting mortalities. Diagnostic testing needs to be quick and reporting by laboratories timely. Appropriate specimens, and proper packaging and transport, are necessary to ensure that there are no unnecessary delays in the delivery of specimens to the testing laboratory. Exclusion of OsHV-1 μ Var can be made initially then exclusions for other diseases completed secondly. Rapid action and response will assist in ensuring greater farmer compliance and cooperation. Under agreed arrangements, testing for suspect exotic diseases should be conducted at AAHL.

Field-based rapid tests do not currently exist but there seems little industry interest in developing such tests. Development of a rapid test would perhaps cost between AUD \$150K to \$450K, take between 1 to 3 years to develop, and would cost approximately AUD \$30 per unit sample. In addition, results from such a test would have to be confirmed at EMAI or AAHL in any event.

Editor's note: Post workshop, information on a LAMP assay (Ren et al., 2010) which has potential as a rapid field test was provided.

New Zealand

Movement in the winter of healthy, but potentially infected, animals occurs throughout sites in the North Island. Only movement of brood stock occurs from the North Island to hatcheries in the South Island.

OIE, EU, EFSA REGULATORY RESPONSES

The disease associated with OsHV-1 μ Var infection is not currently listed by the EU or the OIE as a notifiable disease. In 2010, the European Commission commissioned EFSA to prepare a Scientific Opinion document on OsHV-1 μ Var which included an investigation into causality, an overview of the oyster growing industry, a review of other mortalities and the surveillance activities in place. This paper identified the

significant role French based hatcheries played in supplying spat (both wild caught and hatchery produced) to producers throughout Europe. It also identified key gaps in information including production figures, movement data, health status and husbandry practices. The report discussed environmental factors which might be significant and the possible involvement of several other species. EFSA raised the need for improved biosecurity in hatcheries including the need for certification; improvement in diagnostic methods; the need for a case definition; more data on occurrence and the need for viral strain differentiated epidemiological studies.

In the Council Directives of the European Commission, articles 41 and 43 regulate for emerging and non-listed diseases, respectively. These have recently been reviewed. Commission regulation 175/2010¹⁵ is based on Article 41 and recognises the new genotype associated with increased mortalities. It provides directives for sampling, testing and containment as well as movement restrictions and reporting. A flowchart indicating protocols, in the event of mortalities, for testing, measures to be taken and movement control is also provided.

Decision 2010/221/EU¹⁶ regulates for declarations of freedom from disease and movement restrictions, and amendments to this decision are based on Article 43. As well as providing details on the new genotype, movement restrictions and reporting requirements, this decision offers guidance on the conduct of suitable surveillance programmes. Such guidance will lead to a more harmonised and organised European approach to surveillance for this disease.

The EU is not currently considering listing the disease and is waiting until after April, 2013 to review the question of listing. If the criteria used for listing are consistent with that of other diseases, it is unlikely that OsHV-1 μ Var will be listed. The OIE has not yet discussed listing however it is currently developing (with Tristan Renault) a chapter for its Manual which is to be tabled at the next OIE Commission meeting in October 2011. This chapter will provide diagnostic information and guidance.

The OIE disease listing process is usually instigated by an affected member country. It could be requested by the October 2011 meeting and then may or may not be officially listed at the May 2012 meeting, for example. The OIE process is often seen as slow. The criteria for listing a disease are stated in Chapter 1.1.1¹⁷ and 1.1.2¹⁸ of

¹⁵ <u>http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:052:0001:0013:EN:PDF</u>

¹⁶ http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:098:0007:0011:EN:PDF

¹⁷ http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_1.1.1.htm

¹⁸http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_1.1.2.htm

the *OIE Aquatic Animal Health Code*. The chances for successful listing are reasonably high given the timely nature, the emerging disease category and the fact that the OIE is moving towards listing different pathologies or virus genotypes. The goal would be to collect as much data as possible in support of a request made by a member country. It was noted that member countries from the Southern Hemisphere were keen to be involved in the development of the OIE chapter. A draft of the chapter should be circulated for comment in the October report to members.

The EC regulation (Commission Regulation 175/2010) was seen as weak by some because it only recommended testing in the event of mortalities. When the mortalities stop so, too, does the testing which allows movement to recommence even when in reality the disease may still be present. This weakness in regulations had a potential major impact on all EU states given the reliance on spat from France which could in theory be translocated even in the presence of infection (but without mortalities). The economic impact could be catastrophic. The new Decision 2010/221/EU is based on declarations of freedom from disease and movement restrictions from infected zones or zones for which no demonstration of freedom has been achieved.

THE IMPLICATIONS OF OIE LISTING

The OIE criteria for listing diseases in the *OIE Aquatic Animal Health Code* are included at Article 1.2.1 of the Code. In summary, the criteria cover the following issues:

- Production loss
- Known causal agent or strong association
- Potential for international spread
- Free areas/zones/countries
- Adequate diagnostic tools

These criteria would likely be fulfilled in the case of OsHV-1 μ Var. Classical OsHV-1 is not likely to be listed, because it is ubiquitous. It is interesting to note that it took 8 years for koi herpesvirus disease to be listed.

The implications for listing include:

Positive implications:

• The OIE processes for collecting information on the disease status of member countries would become more complete and timely reporting would provide

broader, more reliable data. The first occurrence of the disease would have to be made within 24 hours of detection.

- Multilateral framework for safe trade of aquatic animals and aquatic animal products with respect to OsHV-1 µVar would be in place instead of bilateral arrangements between individual trading partners.
- Gives a disease a greater profile for public and industry education purposes and may assist funding for research and surveillance activities.
- Having an OIE Reference Laboratory for a listed disease is seen as an enormous advantage.
- Characterisation and diagnostic standardisation: The OIE chapter (even if not a listed disease) will include guidance on diagnostic techniques.
- OIE standards would provide guidance on the establishment of free zones or compartments within affected countries in accordance with OIE criteria.

Negative implications:

- Potential effects on trade of susceptible aquatic animal species and their products. Countries which are disease free may undertake an import risk analysis and make requirements (where there were none previously) of exporting countries for safe trade with respect to OsHV-1 μ Var. Australia has small but increasing oyster exports, and New Zealand exports most of its production so trade restrictions are of considerable importance.
- Not all trade will be impacted the same way. Australia's oyster export trade, excluding pearls and shell, consists of two commodities: (1) Viable spat and (2) Non-viable product (half-shell and meat) for human consumption. The commodity trade that may be most negatively affected would be the emerging trade in viable product i.e. export of spat.

It was noted that some aquaculture regulatory systems, for example, in Southeast Asia are not well connected to the national veterinary systems and this inevitably leads to underreporting of aquatic diseases by Southeast Asian CVOs.

10. PATHOLOGY/NATURAL HISTORY

SPECIES AFFECTED

Classical Herpesvirus is seen in a broad range of molluscs, however, to date, the OsHV-1 μ Var has been associated with disease in Pacific oysters (*C. gigas*) exclusively. Evidence of susceptibility of *Ostrea edulis* (European flat oyster), *Pecten maximus* (scallop) and *Ruditapes phillippinarum* and *R. decussatus* (clam) has been observed. France has detected low level of OsHV-1 μ Var DNA in other species of

mollusc, but there are no signs of viral replication. Other species may act only as mechanical vectors for the virus. Gastropods (sea snails, sea slugs, as well as freshwater snails and freshwater limpets) may be susceptible.

It was recognised that high mortalities in all molluscs should be investigated and tested for $OsHV-1\mu Var$.

DIFFERENCES IN AGE SUSCEPTIBILITY

Data on age susceptibility differ between countries and is perhaps confounded by the rate of oyster growth. In Europe highest mortalities were seen in spat and juvenile oysters, however in Australia and New Zealand adult oysters were also significantly impacted.

ORGANS AFFECTED

Oysters are filter feeders, feeding on naturally occurring plankton and algae. French studies have shown that virus could be detected in the haemolymph and digestive gland after six hours of exposure to the virus. However 72 hours postexposure, levels of virus were highest in the adductor muscle, haemolymph, gills and mantle. A significant increase in the amount of the viral DNA was observed from 72h to 96h post-cohabitation in all analysed tissues, except for the digestive gland.

The dynamics of the virus in oyster tissue will have a major impact on sampling for testing purposes.

NATURAL HISTORY

While there is no confirmed evidence that vertical transmission occurs, it has not been studied sufficiently; vertical transmission is very difficult to assess. It is always possible to find infected larvae, and gonads (not in spat) are infected, suggesting that transmission from adults to larvae is a possibility. Improved understanding of transmission pathways will assist in the possible future development of resistant strains.

Further study may be able to establish if true vertical transmission (gametes are infected and the larvae are infected prior to release) occurs or if it is only pseudo vertical transmission (infection occurs at release or directly afterwards). There is some evidence that vertical transmission does not occur. It was noted that eggs pass through ciliated ducts and could become infected at spawning. Anecdotal reports

from New Zealand suggest that it is possible to produce non-infected larvae from infected oysters.

In cohabitation infectivity trials, the virus was first observed in the mantle, gills, gonads and digestive gland, then later in connective tissue and muscular fibres. It is therefore possible to conclude that waste from infected oysters can be infectious. In addition, the adductor muscle is an important carrier of the virus and, following death, the last part to deteriorate which means moving dead oysters could be risky long after the mortalities have ended.

Infectious virus can be released from live oysters before death and following death. Release of high levels of the virus has been recorded from healthy oysters. Huge levels of viral replication have been seen early in infection and this supports a possible hypothesis for differential infectivity of oysters at different stages.

Histopathology results suggest massive excretion of haemocytes which is a classic immunological response in molluscs. More research could be conducted on the question of apoptosis (programmed cell death), particularly whether OsHV-1 μ Var influences the natural apoptosis response in order to increase its virulence. Examination by transmission electron microscopy (TEM) conducted in France demonstrated apoptosis in cells close to virus-infected cells.

PERSISTENT INFECTION

We know that the virus is found:

- Free-floating (OsHV-1 µVar detected free-floating but may or may not be infectious; it is known that abalone herpesvirus remains infectious for at least 24 hours following release into the water column)
- In survivors of outbreaks (in NSW, survivors show high levels of DNA material a long time after the outbreak)
- In decomposing oyster tissue after death
- In the major organs and tissues of oysters
- In mucus

The virus appears to persist and be able to reinfect in the absence of visible high mortalities which may mean that infected areas may never be able to be cleared of the virus. There is mounting evidence to suggest a latent period where the virus is detectable in healthy oysters. More work needs to be conducted in order to better understand the role of subclinical infection.

11. SURVEILLANCE

WHAT SYSTEMS ARE BEING USED TO LOOK FOR THE DISEASE/VIRUS?

Farmer reporting

Passive farmer reporting is used routinely in all countries and is the single most important and affordable surveillance tool. However, it is also notorious for underreporting. Moreover, there is a general lack of standardization in estimating mortality rates and collecting data related to mortality. Improving the level of reporting of mortalities by farmers is vital for early detection. It must be done intelligently by removing any disincentives and by providing incentives to farmers.

A systematic and collaborative improvement of the passive farmer reporting system could include:

- strengthening communication pathways between farmers, scientists and regulators,
- improving public education,
- reducing laboratory response rates and increasing information provided to farmers regarding their results (particularly if inconclusive),
- providing practical assistance and advice to industry in the event of an investigation,
- empowering farmers to improve their on-farm biosecurity practices by providing practical guidelines,
- strengthening cooperation between growers,
- minimising the impacts of movement or trading restrictions where possible in the event of a disease investigation,
- ensuring pathways for reporting a suspected outbreak are understood by all parties,
- providing important information and feed-back to growers regarding collated data and findings,
- free testing in the event of a suspected outbreak
- providing sampling kits to improve the speed and quality of sample collection for early diagnosis.

Systematic reporting of mortalities should be encouraged including delayed discovery of mortalities. All data should be seen as important and useful to the surveillance of disease. The data can be joined with a wide range of available data collected for other reasons such as past weather or environmental events, stock movement and shipping data, for example, to assist in the modelling of the disease

and assessment of causal agents. The more data that are collected provide a more accurate picture of what constitutes normal and abnormal mortalities. It can also produce important benchmarking data for growers to use in assessing their production systems.

A national oyster farming database would be a useful surveillance tool. Such a tool could be purpose built or an existing information system such as the Tasmanian Pacific Oyster Health Program could be adapted to be made national. Such a reporting system could be developed and conducted through the oyster growing industry itself.

France had a system of reporting mortalities and movements but these reports were incomplete due to low participation by farmers and overshadowed by farmer claims for compensation from the state. Together, these two reporting systems provided only a patchy record of disease occurrence, level of mortalities and spread of disease. Some improvements have since been made.

Sentinel populations in areas free from the disease ('observatories')

As part of follow-up surveillance activities, NSW instigated a system of sentinel sites in the Georges River. Disease-free oysters were deposited in sites in farmed areas and in areas with wild populations. Frequent visits and testing are required and make the activity labour-intensive, however it is seen as a useful tool in early detection. It also avoids the reliance on passive farmer reporting.

Other surveillance activities

The Australian national survey currently being undertaken should provide some much needed information. Pacific oysters only are farmed in South Australia and Tasmania; while Sydney rock oysters, Pacific oysters and Australian flat oysters (*Ostrea angasi*) are farmed in NSW. National surveillance focuses on Pacific oysters only, with farmed populations being a priority (note: wild Pacific oysters are found in NSW & Tasmania). The analysis is to be conducted by AAHL & EMAI using agreed upon methodologies to ensure compatibility.

The UK is currently conducting a surveillance program to demonstrate freedom from disease using a PCR test (the C2/C6 assay) on individual oysters in 13 containment zones. This program is expensive to conduct, however once freedom status is established they will be able to revert to the Passive Farmer Reporting system.



NZ has conducted no surveillance for freedom from disease purposes. They see the costs of attaining and maintaining a free status as prohibitive. The disease is now recognised as being endemic in New Zealand.

Surveillance for the virus/subclinical cases?

There is some evidence of a sub-clinical state of the virus. The following questions were raised:

- Are there different mechanisms of latency?
- Can latent infection be detected with PCR?
- Have exhaustive studies been made of latency markers in genomes?
- How is latent state different from low level, sub-clinical infection?
- Can experimental transmission trials confirm the existence of sub-clinically affected "healthy positives"?
- Is it possible that the virus can be found in a carrier host (low DNA levels detected in other molluscs), infected host (i.e. a Pacific oyster, shedding virus or not) or a reservoir host (harbouring virus in an unaffected population)?

The expert technical advisory group could help in directing research and defining sub-clinical infection questions.

12. RISK FACTOR DATA

RISK FACTORS IDENTIFIED

Several risk factors are recognised as significant, however these factors could be considered relevant for any disease affecting Pacific oysters. The overriding feeling is that once an animal is stressed then its susceptibility to disease is often sudden and profound. Animals might be stressed by over-handling, temperature shocks, rapid growth rates, and the presence of other pathogens such as *Vibrio* spp. for example.

A multifactorial approach is needed when examining causal agents or risk factors. There is a complex web of correlation, for example temperature is an important but not definitive trigger for expression of disease. The different effects of temperature on the virus and on the host make the effect of temperature on the virus-host interaction complex.

The following risk factors have been identified as potentially playing important roles in the complex balance which ensures the good health of an oyster:

Density of oysters

French studies have not found a correlation between density and mortality.

Water temperature

High mortalities associated with the virus have also been associated with higher water temperatures. Patterns of mortalities seen in France and New Zealand have indicated a close connection to a rise in water temperature rather than spread of a pathogen, indicating that the pathogen may have been widespread and disease was expressed due to environmental factors. Simultaneous outbreaks have occurred in various parts of the North Island of New Zealand and in France with no obvious connection between sites. The presence of the disease has been recorded in a range of water temperatures between 13°C and 23°C. Mortalities in New Zealand occurred after a sudden rise in water temperature (3°C in one week). Larvae can stay in the water column for over three weeks and are able to travel over great distances with the prevailing water currents, however the simultaneous occurrence of mortalities in sites around the North Island and not the South Island suggests that infected larvae are not the sole cause of these outbreaks.

The absence of unexplained high mortalities in cooler oyster growing areas, for example, the South Island of New Zealand, and Scotland, may suggest that water temperatures are too cold in these locations for the virus to cause disease even if it is present.

Salinity

There is no evidence to suggest that salinity plays any important role.

Water quality

Poor water quality (a brown plume) around Woolooware Bay was reported prior to the outbreak in November 2010. Stock moved from Woolooware Bay to the nearby, more protected Quibray Bay one week after the outbreak did not show mortalities until six weeks after translocation. Water quality testing in Woolooware Bay at the time of the outbreak (but sometime after the report of poor water quality) showed no significant issues.

Oysters are often grown in coastal areas that may have naturally variable water quality. They may also be adjacent to areas of high urban density, shipping harbours, or close to industry or agriculture that could affect water quality.

France found no causal association with poor water quality and infection however agreed that poor water quality may prompt stress and thereby make infected animals more susceptible to disease.

Ireland has anecdotal or scant evidence that suggests differential mortalities in different bays associated with water quality, but differences in mortalities between producers within the same bay were observed. Production techniques and spat source are very similar in France and Ireland.

Age or Growth rate

In New Zealand, where most oysters are grown from wild spat, the larger oysters from wild stock appeared to die first. However, 90–100% mortalities were seen in hatchery spat. In France, mortalities were seen in all age groups but mostly in spat. In Australia, mortalities were seen in all age groups.

Management practices

Good management practices can assist in keeping oysters healthy and allow oysters to develop (rather than inhibit) strong generic mechanisms for resisting disease. But what constitutes "good management practices" exactly? Drying equipment, keeping good records, not moving sick stock, reducing stress are some important factors.

Proximity to infection

Diseases caused by other aquatic Herpesviruses, in pilchards for example, have been modelled and it was shown that close contact was required for transmission to occur. In addition, Ifremer is developing a model to understand spread of infection in a bay including both hydrodynamic and oyster transfer aspects.

Depth of oysters or tidal location

Research is beginning to suggest that oysters which are in contact with the water column the longest (e.g. sub-tidal) experience the highest mortality rates and oysters in intertidal zones experience lower mortalities. This observation could be confounded by different growth rates, which are higher for animals fully immersed. Oysters out of the water and thus kept closed for longer periods necessarily feed less and grow more slowly. They also display a natural anaerobic resistance to disease. Some evidence suggests that oysters in intertidal zones become adapted to the temperature changes that occur more frequently in shallower areas and are less stressed by temperature shocks than are constantly immersed oysters. New Zealand producers using long-line systems (at depths of 6–8 metres) appear to be as affected as farms at other depths.

Presence of other pathogens

Work in France on affected oysters found *Vibrio* coexisting with the virus in many cases. Infection with *Vibrio* or other pathogens would probably make oysters more susceptible to other infections.

Shipping and equipment movements – including biofouling, ballast water etc.

Recently imported, used aquaculture equipment was ruled out as a possible entry point of the virus in the case of the Georges River outbreak. Survival of infectious virus of outside the host is thought to be low. Movement of Pacific oysters and other potential host organisms on ship hulls (biofouling) was considered a more likely possible mechanism for transmission than ballast water, given the low survival rate of the virus outside its host.

Singapore (a major source of ships to Australia) farms Pacific oysters in their shipping harbour, however there have been no reports of disease. However, it is not known whether an OsHV-1 epizootic would be detected and reported in Asia if it were to occur.

Genetics

It is not clear whether all *C. gigas* are in fact the same. Studies using selected family lines suggest some lines show marked differences in prevalence of infection. Standardised infection models would assist in determining family susceptibility differences and this would inform any future development of breeding programmes for resistant oyster lines. Several states and organisations in Australia have the capacity to undertake this work, with SARDI, EMAI and USyd expressing an interest to do so.

The development of resistant lines may take a very long time to develop however; selective breeding in the Sydney rock oyster took 12–15 years but the generation times are recognised as being much longer for this species. Between-line and within-line selection and breeding from surviving oysters could be considered.

France has developed a more resistant strain which has shown reduced mortalities.

SYSTEMS TO MEASURE RISK FACTORS

Baseline, mortality and environmental data collection and analysis

The routine collection of important baseline data is crucial. Even basic population data are weak and are only estimates. South Australia claims to have very accurate centrally-located data which are readily available. New Zealand has no data on numbers of animals and has only recently collected basic farm data. New Zealand authorities have found movement data very difficult to collect. NSW records how many triploid spat are in the state and on an estuary-by-estuary basis, including what leases they are on and which farmers have them (in NSW, Pacific oysters grow quickly and are replenished regularly - close to annually). Privacy issues and poor

data quality are important and sometimes problematic issues which hamper the sharing or use of any data.

It was suggested that an industry-run national database might avoid some of the privacy issues and may encourage improved data quality. Data could be entered at the lease-side via a digital diary system, for example, collecting data on all routine management activities as well as animal health, water quality and other environmental factors. Data would then be immediately available for analysis and would provide feedback with relevant benchmarking and industry averages to assist farmers in improving management strategies.

- Mortality reports
 - Underreporting of mortalities could weaken any analysis (see Passive Farmer Reporting). A voice recognition system to collect movement data should soon be in operation in NSW. Integration between data sources is important and the benefits of any system need to be demonstrated to producers in order to ensure their cooperation.
- Environmental monitoring, including
 - o Hydrodynamic modelling
 - o Standardised water quality and temperature measurement
 - Environmental data loggers could be used and would be able to feed information directly into an industry database for real-time epidemiological analysis.
- Measuring husbandry factors
- Movement and traceability

Pre-season preparation of risk factor survey

It was suggested that a survey be designed in preparation for summer 2011. This could be put in place in readiness for any possible further outbreaks that might occur in Australia. The survey would provide an opportunity to use current knowledge to measure identified risk factors and the presence of disease. The results could be extremely informative to all stakeholders and may influence management practices, response, husbandry, testing, institutional systems etc. Such a survey could be useful in informing for other diseases as well. Funding limitations were recognised as being a hurdle.

Experimental Infection Modelling

Development of experimental infection models has been undertaken in France and in Australia. Such models will facilitate further research on the biology of this virus.

13. CONTROL

WHAT IS BEING DONE? IS IT WORKING? WHAT IS PROPOSED?

It was acknowledged that industry is seeking practical measures which they can rely upon and adopt, however it is probably inappropriate to be too prescriptive because control measures will be site-specific or farm-specific depending on a range of management, environmental and other factors.

The following activities/issues were reported during discussion:

France

- Undertaking breeding programmes for oysters with disease resistance.
- Have tried earthen ponds for finishing oysters (claire system where oysters are kept for several days to several weeks for fattening), which is proving useful in reducing mortalities. No mortalities seen.
- Suggesting the production of alternative oyster species which are resistant (may also dilute the virus) for diversification purposes.
- French farmers want to experiment with *C. gigas* from other countries.
- Placing resistant strains in strategic locations in estuaries in order to promote resistance in the wild populations
- Looking at exposing spat to infection in hatcheries in order to make them less susceptible when subsequently re-exposed to OsHV-1 μ Var in the field (to confirm the observation that naïve oysters are more susceptible to infection than previously exposed oysters).

New Zealand

- Some farms stopped moving and grading oysters, for example, to reduce stress caused by handling, particularly during summer. Decreased mortalities were observed, presumably due to the reduced stress levels in animals. The gene expression of noradrenaline suggests stress during handling.
- Conducting small scale experiments on density and water depth

Anecdotal evidence and other suggestions include:

- "Overstocking" to maintain production goals but this is potentially a very expensive option for those farms dependent on expensive spat from hatcheries.
- The seeding of resistant oysters into areas which contribute most to natural recruitment
- Hatchery producing certified $OsHV-1 \mu Var$ free spat. However it was noted that it was not necessarily a good strategy to put naïve spat into infected areas.
- Selling early as an emergency, pre-emptive response.

New South Wales

- Growing other species such as the Sydney rock oyster or others to diminish the infection load.
- Work on 20 family lines indicated marked differences susceptibility. A field trial in Georges River indicated marked differences in infection rate between families. These results indicate that there may be potential for further work. Accessing survivors from infection trials for breeding in a clean hatchery is problematic. However, these are pedigreed lines and full-siblings of each of the lines are available from OsHV-1 free estuaries elsewhere for breeding purposes.
- A standardised infection model may assist with testing different families. However standardising exposure to look at interaction of both genetics and environment was acknowledged as difficult. Several states and organisations in Australia have the capacity to undertake infection trials, with SARDI, EMAI and USyd expressing interest to do so. FRDC is currently considering funding further research in the area.

14. GROUP DISCUSSION

Towards the end of the second day, workshop participants were asked to break into three groups representing the oyster growing industry, scientists, and regulators. Groups were asked to identify the areas of key interest, knowledge gaps and research priorities.

See Annexe 4 for notes on these discussions. <u>Summaries of key findings</u>, <u>knowledge gaps and research priorities</u>

15. SOME PRESENT AND FUTURE RESEARCH ACTIVITIES

OSTREID HERPESVIRUS-I (OSHV-1) RESEARCH AT THE ELIZABETH MACARTHUR AGRICULTURAL INSTITUTE

Pacific oyster mortality associated with Ostreid herpesvirus-1 μ Var (POMS) was first diagnosed in Australia in December 2010 at Elizabeth Macarthur Agricultural Institute (EMAI). Since then scientists at EMAI have been engaged in diagnostic activities (virology, histopathology and EM) during investigations of oyster mortality cases. The Virology Laboratory has also provided full laboratory support for a state-wide survey and detailed studies of infected populations. Sample collection was undertaken by NSW DPI staff. Collectively, at the laboratory these activities have involved the collection and testing of samples from more than 4500 individual oysters in a period of just over 6 months. Considerable research has been undertaken to support these activities, as outlined below.

- 1. Development, optimisation and validation of diagnostic assays: A highthroughput capacity for testing oyster tissues for OsHV-1 using real-time polymerase chain reaction (qPCR) assays was developed. This enabled rapid results to be provided in cases in which POMS was suspected. The procedure included optimised tissue selection, collection and sample preparation methods to provide purified DNA for testing in the qPCR. The qPCR assay was considered by EMAI to be 'fit for purpose' under Australian conditions and was shown to be superior to existing methods, including a published qPCR. A suite of additional molecular techniques targeting multiple portions of the OsHV-1 genome and utilising different PCR platforms were concurrently developed. These additional methods were utilised to distinguish active viral replication from potential environmental contamination and to confirm the OsHV-1 infection status of suspect samples. These assays provided valuable diagnostic and research tools which have already been broadly applied.
- 2. Investigation of disease outbreaks: Additional resources have been devoted to investigating cases of oyster mortality throughout NSW. In addition to determining the presence of OsHV-1 infection, the roles of other pathogens and environmental conditions have been determined. The aim is to assist the oyster industry by improving general diagnostic capacity and to generate the epidemiological data required to adequately manage the threat of POMS.

- 3. Pathogen surveillance: As part of the national response to POMS the Aquatic Consultative Committee on Emergency Animal Diseases (AqCCEAD) instigated a survey to determine the distribution of OsHV-1 μVar in wild and farmed Pacific oysters in Australia. Testing at EMAI supported this survey and enabled the scope of the survey in NSW to be extended to include Sydney rock oysters and to distinguish the OsHV-1 reference strain from OsHV -1 μVar.
- 4. Pathogenesis and epidemiological studies: In the course of investigating POMS outbreaks in the Georges and Parramatta Rivers, samples have been collected from cohorts of oysters representing different age classes, species and exposure times. These have included survivors of outbreaks as well as newly recruited wild spat and oysters translocated into the area specifically for the purpose of pathogenicity studies. These samples have been subjected to a range of techniques including histopathology, bacteriology, quantification of viral loads by qPCR, viral sequence determination, *in situ* hybridisation and electron microscopy. High quality images of the Australian strain of OsHV-1 were obtained during these studies – the first occasion on which the virus has been visualised. Valuable data are being compiled which will assist in management and control of POMS in the future.
- 5. Investigation of genetic resistance to POMS: Preliminary research using 20 different Pacific oyster family lines has given promising results, with some lines almost completely resistant to infection and indicates that genetic resistance to OsHV-1 is likely to provide a practical response to the threat of this disease. Additional trials are currently underway in collaboration with NSW Department of Primary Industries, Fisheries staff.

FRDC INDUSTRY SUPPORT PROJECT

Understanding and planning for potential impacts of OsHV-1 μVar for the Australian Pacific oyster industry

- 1. Collate industry relevant information both published and anecdotal
 - Oyster Herpes Virus Workshop, July 9-10, 2011, Cairns, Queensland
 - International Oyster Symposium Sept 15–18, 2011, Hobart, Tasmania
 - Lessons learned from previous incidents
 - Other sources
- 2. Field Trip to visit production sites in France and Ireland

- Meet and share experiences with farmers in these affected countries.
- Probably should be prior to November.
- Share findings with the Australian industry.
- 3. Develop and Communicate
 - Strategies to minimise risk
 - Response activities and long-term planning
 - Strengthen control measures where disease is established
 - Inform regulators
 - Information sharing

ANNEXES

ANNEXE 1: WORKSHOP PARTICIPANTS

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ANNEXE 2: WORKSHOP PROGRAM

Time	Session	Discussion points
Saturday 9	9 July 2011	
9:00	Introduction	Introduction of organisers, facilitators and participants Objectives Agenda Outputs
9:30	The disease	What is the disease? What is the cause of the disease? - How strong is the evidence? - Are other pathogens involved? Case definition?
10:00	Emergence and distribution	Where is it? Emergence and distribution in Europe Emergence and distribution in Australia and New Zealand Evidence from other parts of the world?
10:30	Break	
11:00	Emergence and distribution	Continued
12:00	Impact	Impact in different locations Mortality levels observed Scale and importance of the disease Current impact and potential future impact Zoonotic potential?
13:00	Lunch	
14:00	Characterisation	What is it? Relationship to non-variant OsHV-1 - Viral population shifts (proportion of μ var and traditional viruses isolated over time) - Stability of the virus
15:00	Diagnostics	Current options Case definitions (suspect and confirmed) Tests available - Clinical signs - Gross pathology - Histology - Immunoassays - Molecular assays Validation of tests, estimates of test characteristics (Se and Sp)
15:30	Break	

Time 16:00 17:00	Session International response and regulation	Discussion points OIE, EU, EFSA, National regulatory responses. Are these appropriate, are they well founded, are they enough? OIE - is this going to become a listed disease? - if so, what are the implications? European, Australian and New Zealand regulatory responses - Reporting requirements? - Internal movement restrictions? - Internal movement restrictions? Implications for non-affected countries
17100		
Sunday 10	July 2011	
9:00	Pathology / natural history	Species affected Differences in age susceptibility Organs affected Natural history Persistent infection? Transmission - vectors?
10:00	Surveillance	What systems are being used to look for the disease / virus? Surveillance for mortalities - systems used in different countries Farmer reporting - mortalities or claims for compensation? Sentinel populations ('observatories') Surveillance for the virus / subclinical cases?
10:30	Break	
11:00	Epidemiology	Environmental factors - Seasonal effects - Temperature - Water quality - Management factors Transmission and spread - Role of hatcheries - Role of movements
11:30	Risk factor data	Available systems to measure risk factors - Environmental monitoring - Measuring husbandry factors - Movement and traceability
12:00	Control	What is being done? Is it working? What is proposed? Immunity and resistance: vaccination, chemotherapy, immune-stimulation, resistance breeding, restocking with resistant species Sanitary measures: blocking agents, disinfection of eggs and larvae Husbandry practices: stocking density, depth, movements
13:00	Lunch	
14:00	Overview of current knowledge	Summary of key findings

Time	Session	Discussion points
15:00	Identification of key knowledge gaps	Objectives: - Prevent further spread, - Manage the disease in areas where it already exists to minimise impact, - Consider options for eradicating the disease from populations Do we have enough information to achieve these objectives? List of key questions that still need to be answered Key systems and requirements that are not in place
15:30	Break	
16:00	Planning research priorities and opportunities	Review of current research activities and capabilities Identification of priorities and opportunities: - Research - Management - Contingency planning
17:00	Workshop close	

ANNEXE 3: SUPPORTING DOCUMENTS SUBMITTED AT WORKSHOP

OSHV-1 µVAR OUTBREAK IN WHITSTABLE BAY, UK. ED PEELER, CEFAS.

Descriptive epidemiology of the outbreak

The Fish Health Inspectorate (FHI), Cefas were contacted by Seasalter Foreshore oyster farm on 12 July 2010. The farm reported unexplained mortality in Pacific oysters (*Crassostrea gigas*) (first observed on 8 July 2010). A visit was made by a FH inspector on 13 July. The farm is location in the Thames estuary.

Seasalter Foreshore oyster farm is located on the western edge of Whitstable and is owned by John Bayes who also runs Seasalter hatchery (located approximately 15 km east of the foreshore site at Reculver). The site has been leased to a French company since early this year and stocked with 8 million juvenile oysters from the Seasalter hatchery. The site is largely operated by French staff. Oysters are grown in bags on trestles in the tidal zone.

Mortality varied from 40-90% between batches. All age groups were affected. Oysters higher up the beach (submerged for a shorter period) were reported to be less affected (consistent with observations of higher mortality in oysters submerged for longer periods in the Republic of Ireland). The water temperatures when the mortality occurred were the highest recorded that year (>20°C). Sewage had been released into the vicinity of the sites on 8 July which may have resulted in decreased dissolved oxygen levels.

30 oyster samples were taken from both the affected site and Seasalter hatchery. PCR positive results for OsHV-1 μ var, confirmed by sequencing, were obtained from 26 of the 30 oysters from Seasalter Foreshore site (which will be referred to as the index site). The hatchery sample tested negative. A further 150 oyster sample has been collected from the hatchery but were found to be negative (later sample also tested negative, the hatchery distributed stock to other sites which have all tested negative and there were no reports of mortality).

Cockles and mussels are harvested in the Thames estuary. Native oysters are also present although current levels do not support a significant commercial fishery. Wild beds of *C. gigas* exist in the vicinity of the site. Subsequent testing of wild stocks proved negative except for 3 of 30 wild *C. gigas* which tested PCR positive for the OsHV-1 µvar.

Assessment of routes of introduction of OsHV-1 µVar to Whitstable Bay

A range of routes were identified. Two routes stood out clearly as the most likely routes of introduction:

Introduction of materials (e.g. trestles and bags) from France

The company operating the affected site has brought equipment (trestles and bags) from France. The owner of the affected site claims that the bags had been out of the water for 4 years before being shipped to the UK thus no shellfish should have been accidently transported. The trestles had been stored out of water for longer. The equipment was second hand, and the owner said that as well as being encrusted with acorn barnacles there was empty shell within the bags.

Oysters from Jersey (via the Whitstable Oyster Company)

The Whitstable Oyster Company operates a fishery for oysters, two quayside restaurants and a small area of trestles for keeping oysters in seawater. It is known that they purchased oysters which had originated from Jersey and had been depurated at Maldon, Essex (a site authorised by Cefas for this trade). These oysters may have been kept in tanks at the purification centre operated by the company from which water had been discharged (untreated) into Whitstable harbour. Secondly, there is a possibility that oysters purchased from Billingsgate or from the purification tanks may have been relaid on trestles opposite one of the restaurants. Relaying depurated oysters is illegal.

Background

There is an export trade in live Pacific oysters for on-growing from France to other parts of Europe. In 2009 reports of extensive mortalities of oysters were received from the Republic of Ireland (D. Cheslett, pers. comm.) and from Jersey (M. Gubbins, pers. comm.). The presence of OsHV-1 μ Var1 was confirmed in samples from both the RoI and Jersey, and in both cases, the oysters originated in France. There is an increasing amount of circumstantial evidence from areas where mortalities are occurring that infection can be transmitted from non-clinically affected surviving adult oysters to naive juvenile oysters. (F. Geoghegan, pers. comm.).

In Ireland high mortality and the presence of OsHV-1 μ Var were reported from oyster growing sites in 16 bays (D. Cheslett pers. comm.). Pacific oysters are cultured in 44 bays in the RoI, of which 21 introduced spat during 2009. Oysters from France had been imported during 2008 or 2009 to all but one of the bays where

OsHV1 μ Var1 was detected (the other site had introduced oysters from another bay in the RoI which was OsHV-1 μ Var positive). Anecdotally the level of mortality varied considerably between sites within the same bay.

Questionnaire study

A retrospective questionnaire survey of 70 oyster farmers was undertaken to investigate the distribution and determinants of the mortality. Based on farmer recall, mortality data at the batch level were recorded: cumulative mortality, duration of the mortality event, age of animals affected, date of introduction. Observable mortality was recorded in 109 of a total of 346 batches from 47 sites, 104 of the 109 batches were located in bays where OsHV-1 µVar had been detected. The records from bays where OsHV-1 µVar had been detected were analysed to characterise the pattern of mortality and potential risk factors. The mean batch mortality was 37% (18-65% inter-quartile range) but showed a bimodal distribution (half the batches had mortality less than 45%). Mortalities started at the end of May and continued until early August, peaking in early July. On average oysters died over a period of 18 days. There was considerable variation in mortality both between and within bays. Mortality started in batches introduced within the last 12 months and occurred later in the season in established oysters, which is consistent with the introduction of an infectious causative agent. Mortality was significantly higher in spat than other age groups, which supports observations from France. There was a strong association between triploidy and higher batch level mortality: 21% of triploid batches experienced >40% mortality compared with 10% of diploid batches (P <0.01, χ^2 = 10.54, n= 293). The apparent susceptibility of triploid stock may be attributable to their increased growth rate, compared with diploid stock. No batch which was out of water for more than 8 hours during the tidal cycle suffered mortality higher than 40%. Again this correlation may be explained by growth rate; oysters which are immersed for longer grow faster. Manual, compared with mechanical handling, of sacks is associated with higher levels of mortality in spat (~80 versus ~50%); the most likely explanation is that handling provides greater opportunity to record mortality. Future studies should develop improved methods to assess oyster mortality and follow stocks over time to better determine the influence of management and environmental factors on mortality.

At Cefas we have compared the sensitivity of three assays available for the detection of OsHV-1 and the OsHV-1 μ Var. Preliminary results are discussed below.

The four assays compared are:

- 1. Conventional
- 2. Nested PCR

The conventional PCR was performed using the C2 and C6 primers. The nested PCR was performed as above using primers OsHV-1 for and OsHV-1 rev and the C2/C6 reaction product as a template.

3. Sybr green real-time PCR assay

The sybr green assay was that described by Webb et al (2007) using primers OsHVDP for (ATTGATGATGTGGATAATCTGTG) and OsHVDPrev (GGTAAATACCATTGGTCTTGTTCC).

4. Taqman real-time PCR assay

The Taqman real-time PCR used was that described by Martinot et al (2010) using primers OsHV1BF (GTCGCATCTTTGGATTTAACAA) and B4 (ACTGGGATCCGACTGACAAC) and probe (FAM TGCCCCTGTCATCTTGAGGTATAGACAATC TAMRA).

The Taqman and nested assays proved most sensitive, and were able to detect the virus in the sample when diluted a further 1:10 to 1:100. When using DNA extracted from the low level infections the conventional and SYBR green assays detected the virus in undiluted samples only.

ASSOCIATION BETWEEN OSHV-1, OSHV-1VAR AND OSHV-1 μ VAR AND MOLLUSC MORTALITIES. NICK MOODY, AAHL.

Herpes-like viruses have been described in molluscs since the 1990s (see references for general information in the Introduction). The identification of the viral particles in affected oysters as herpes-like viruses was by TEM.

With the advancement of molecular tools, in particular PCR, assays were developed which provided more detail of the genomic characteristics of the viruses which were present. PCR tests were developed to target the A, B and C regions of the ~207kb dsDNA genome (Arzul et al., 2001a). These reported the detection of OsHV-1 associated with mortalities in juvenile C. gigas, and R. descussatus and from healthy O. edulis in France from samples obtained between 1995 and 1999. They also reported the detection of a variant form of OsHV-1 (OsHV-1Var) associated with mortalities in juvenile C. gigas, and R. philippinarum. The variant produced a smaller amplicon using the C2/C6 primer set and no comparative sequence information was provided on amplicons generated from the A or B genomic regions. The variant contained several single nucleotide substitutions and a deletion of 200bp near the C2 sequence as well as an insertion of 27 bases (Arzul et al., 2001a). Additional PCR testing identified a 2.8kb deletion in OsHV-1Var in the inverted repeat region. In 1991, OsHV-1Var was also reported in larval *P. maximus* associated with mortalities in France (Arzul et al., 2001a). In 2002, OSHV-1 was reported from asymptomatic adult C. gigas in France (Arzul et al., 2002).

In the USA, repeated summer mortality events in cultured *C. gigas* occurring during 2002 and 2003 were investigated using the Arzul et al. (2001a) OsHV-1 A, B and C primer sets. Presence of OsHV-1 was confirmed by sequencing of the amplicons however as no amplicons were produced using the C primer set, no discrimination between OsHV-1 and OsHV-1Var was made (Friedman et al., 2005). A review of OsHV-1 in 2007 determined that infection in juvenile bivalves is more likely to result in disease than infection in adult bivalves and that OsHV-1 and OsHV-1Var are considered representatives of a single viral species (Batista et al., 2007).

Investigation of healthy oysters from Asia identified OsHV-1 in *C. ariakensis, C. siakmea, C. gigas* and *C. hongkongensis* using the Arzul et al. (2001a) OsHV-1 A primer set, however differences in sequences were limited to single nucleotide polymorphisms and detailed sequence comparisons were not presented (Moss et al., 2007). These authors described 2 genetic strains in Japan, 1 in Korea, and 2 in China, and suggested sequencing of additional gene regions to further characterize the differences.

In 2010, an additional variant was reported (Segarra et al., 2010). These authors tested larval *C. gigas* obtained after mortality events in France in 2008 and identified both OsHV-1 and a third genotype, OsHV-1 μ Var. The OsHV-1 μ Var differed from OsHV-1 by a single addition, several substitutions and deletions. In one batch of samples both OsHV-1 and OsHV-1 μ Var were detected. Unfortunately, while sequence comparisons were undertaken between OsHV-1 and OsHV-1 μ Var, no sequence comparisons were made with OsHV-1Var. Segarra et al. (2010) found that both OsHV-1 and OsHV-1 μ Var were associated with mortality events in 2008 and there was no relationship between geographical location and virus genotype. These authors proposed additional work to fully investigate the possible infectivity and virulence differences between the OsHV-1 and OsHV-1 μ Var genotypes.

The EU issued a Regulation relating to OsHV-1 μ Var in 2010 (EU Commission, 2010) which requires testing for detection/absence of OsHV-1 μ Var when increased mortality in *C. gigas* is reported. This is required as there are still great uncertainties regarding the emerging disease situation.

Summary

- OsHV-1, OsHV-1Var and OsHV-1 μVar have been associated with disease in young *C. gigas*.
- OsHV-1 and OsHV-1Var have been associated with disease in young *C. gigas* and *R. philippinarum*
- OsHV-1 has been associated with disease in young *C. gigas* and *R. descussatus*.
- Only limited comparative testing has been undertaken, primarily by French scientists. Reports in the literature either do not use the C2/C6 primer set, which enables discrimination between OsHV-1, OsHV-1Var and OsHV-1 µVar, or very limited if any sequence analysis is undertaken.
- More research is required, targeting different regions of the OsHV-1 genome to enable detailed comparisons between the reference and variants strains.
- Infectivity trials are required to enable any virulence comparisons between the reference and variant OsHV-1 genotypes.
- There is one full OsHV-1 genome sequence and one C2/C6 OsHV-1 genome sequence in the public domain (GenBank) so comparisons of the Australian OSHV-1 sequences with exotic reference OsHV-1 and variants are very limited.

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Association between OsHV-1, OsHV-1Var and OsHV-1µVar and mollusc mortalities

Date Location	Species	Mortalities		Detection Method				Deference		
	Location	Species	wortaittes	TEM	PCR A	PCR B	PCR C	Analysis	Reference	
1995-	France	O. edulis (n=3)	No	+	+	+	+	OsHV-1	Arzul et al (2001) Evidence for interspecies	
1999		C. gigas (n=3)	Yes	+	+	+	+	OSHV-1	transmission of oyster herpesvirus in marine bivalve. J	
		R. descussatus (n=3)	Yes	+	+	+	+	OSHV-1	Gen Virol 82: 865-870	
		<i>C. gigas</i> (n=18)	Yes	+	+	+	-	*OsHV-1Var		
		R. philippinarum (n=3)	Yes	+	+	+	-	*OsHV-1Var		
Larval sam	Larval samples tested									
PCR A: A3	/A4, PCR B: B1	/B2, PCR C: C1/C6								
*OsHV-1V	ar only detect	ed in the 2 species in a singl	e hatchery durin	ig one ep	isode of m	ortality				

Data	Data	Species	Mortalities	Detection Method				Sequence	Reference
Date Location	Species	wortailties	TEM	PCR G	PCR B	PCR C	Analysis	Reference	
2000	France	P. maximus (n=4)	Yes	Yes	+	+	+	OsHV-1Var	Arzul et al (2001) French Scallops: A New Host for Ostreid Herpesvirus-1. Virology 290: 342-349
	iples tested 3/Gp4, PCR B: B3/	I /B4, PCR C: C2/C4.	1	I					030 Clu Helpesvilus 1. Vilology 250. 542-545

Data	Crasica	Martalitica	Detection Method				Sequence	Reference	
Date	Location	Species	Mortalities	ISH	IHCT	PCR B	PCR C	Analysis	Reference
2000	France	<i>C. gigas</i> (n=30)	No	+	+	+	+	OSHV-1	Arzul et al (2002) Detection of oyster herpesvirus DNA and proteins in asymptomatic <i>Crassostrea gigas</i> adults. Virus Res 84: 151-160
Adult sam PCR B: B3/	ples tested 'B2, PCR C: C2/C6								

Data	Location	Crossies	Mortalities		Detectio	n Method		Sequence	Reference
Date Location	Species	wortaittes	Histo	PCR A ₁	PCR A ₂	PCR C	Analysis	Reference	
2002- 2003	USA	C. gigas	Yes	+	N/A	+	-	*OsHV-1	Friedman et al (2005) Herpes virus in juvenile Pacific oysters <i>Crassostrea gigas</i> from Tomales Bay, California, coincides with summer mortality events. Dis Aquat Org 63: 33-41
Mortalitie PCR A ₁ : A3	Larval and juvenile samples tested Mortalities associated with elevated water temperatures PCR A ₁ : A3/A4, PCR A ₂ : A5/A6 (nested PCR), PCR C: C2/C6 *No PCR C positive material so unable to determine if it was the variant								

NOTES from Batista et al (2007) Detection of ostreid herpesvirus 1 DNA by PCR in bivalve mollusks: A critical review. J Virol Methods 139: 1-11

- Viral infections are have been observed in adult bivalves but adults are apparently less sensitive to such infections compared to younger stages
- A variant of OsHV-1 (OsHV-1var) was also described in larvae of different bivalve species and OsHV-1 and OsHV-1var are considered representatives of a single viral species.
- The C region encodes parts of two proteins of unknown functions and is present twice in the genome (being located in the inverted repeats TR_L and IR_L).
- The differences observed in herpesvirus DNA detection suggested that the one-round PCR with the C2/C6 primer set was more useful for epidemiological surveys than the nested PCR using the A3/A4 and A5/A6 primer set.
- No amplification of OsHV-1var DNA with the C1/C4 and C1/C6 primer set, smaller amplicons produced with the C2/C4 and C2/C6 primer sets. Identical sized amplicons for both OsHV-1 and OsHV-1var using the A2/A4, B1/B2, B2/B4 and Gp3/Gp4 primer sets.
- C2/C6 allows differentiation of OsHV-1 and OsHV-1var but failed to amplify OsHV-1 detected in the USA.

Data Location	Creation	Mortalities	Detectio	on Method	Sequence	Reference	
Date	Date Location	Species	wortanties	PCR A ₁	PCR A ₂	Analysis	Reference
2007	China Japan Korea	C. ariakensis C. siakmea C. gigas	No	N/A	+	*OsHV-1 (SNP differences	Moss et al (2007) Pathogens in <i>Crassostrea</i> <i>ariakensis</i> and other Asian oyster species: implications for non-native oyster introduction to
		<i>C. hongkongensis</i> /A6 (nested PCR) A region: 2 genetic stra				in the A region)	Chesapeake Bay. Dis Aquat Org 77: 201-223

Data	Location	Species	Mortalities	Detection Method			Sequence Analysis	Reference
Date	Location	Species	wortaittes	PCR A		PCR C	Sequence Analysis	Reference
1995-	France	C. gigas (32 isolates)	Yes	+		+	OsHV-1	Segarra et al (2010) Detection and description of a
2007								particular Ostreid herpersvirus 1 genotype associated
								with massive mortality outbreaks of Pacific oysters,
2008	France	C. gigas	Yes	+		+	17 OsHV-1	<i>Crassostrea gigas,</i> in France in 2008. Virus Res 153:
							15* OsHV-1µVar	92-99

PCR A: IA1/IA2 (new PCR primers), PCR C: C2/C6

* OsHV-1µVar different to OsHV-1Var

- In one batch, both OsHV-1 and OsHV-1µVar were detected. Sequence comparison between OsHV-1 and OsHV-1µVar but no sequence comparison with OsHV-1Var
- No relationship between geographical location and virus genotype
- Both OsHV-1 and OsHV-1 μ Var associated with mortality events in 2008
- Important information attributed to (data not shown) and (...., personal communication)
- More work is need to fully investigate the possible infectivity and virulence differences between the OsHV-1 and OsHV-1µVar genotypes
- Detection of the two genotypes in some samples collected in 2008 led suspect (sic) the presence of both genotypes in one individual. A study using the cloning technique has been carried out to investigate this aspect but has been inconclusive (data not shown).
- Our work revealed the emergence of a third genotype, OsHV-1µVar, associated with abnormal mortalities of *C. gigas* in France.

Date	Location	Species	Mortalities	Sequence Analysis	Reference
2008	France	C. gigas	Yes	OsHV-1	COMMISSION REGULATION (EU) No 175/2010
	Ireland			OsHV-1µVar	of 2 March 2010 implementing Council Directive 2006/88/EC as
					regards measures to control increased mortality in oysters of
2009	Ireland	C. gigas	Yes	Suggestion OsHV-	the species Crassostrea gigas in connection with the detection
	United Kingdom			1µVar played a role	of Ostreid herpesvirus 1 μvar (OsHV-1 μvar)

• When increased mortality in *C. gigas* is reported, testing for detection/absence of OsHV-1µVar should be carried out

• The availability of accurate and timely information on the situation as regards the detection of OsHV-1 µvar in the Member States is a key element to ensure a proper control of the emerging disease situation. For that purpose, Member States should inform the Commission and the other Member States of the first confirmed presence of the OsHV-1 µvar virus on their territories in 2010 without undue delay.

- As there are, the measures provided for in this Regulation should apply until the end of December 2010.
- For the purposes of this Regulation, OsHV-1 μvar means a genotype of the virus Ostreid herpesvirus-1 (OsHV-1) which is defined on the basis of partial sequence data exhibiting a systematic deletion of 12 base pairs in ORF 4 of the genome in comparison with OsHV-1 (GenBank # AY509253).
- The following primers must be used: CF and CR (These primers or descriptions thereof may be obtained from the Community Reference Laboratory for Mollusc Diseases (LGP-Ifremer, av de Mus de Loup, 17390 La Tremblade, France). The presence of OsHV-1 µvar in a sample is indicated by the presence of a band of the appropriate size (157 bp instead of 173 bp for OsHV-1) on a 2.5 % agarose gel with all negative controls negative and all positive controls positive.

ANNEXE 4: GROUP DISCUSSION RESULTS

SUMMARIES OF KEY FINDINGS, KNOWLEDGE GAPS AND RESEARCH PRIORITIES

GROWERS

Key findings

- Growers agreed that the similarity between the virus genotypes found in Australia and New Zealand and those in Asia was a surprising result considering that no reports of mortalities associated with herpesvirus or even unexplained mortalities had been made from Asia. Given the importance of shipping routes between Australia and Asia it seems unusual that there have been no reports of suspected occurrence of the disease in Asia.
- The issue of water temperature limits is confusing. Research first suggested 18° C was the lower limit but with further research this estimate has changed to 16° C then 14° C. It was noted that this change in temperature limit could be misconceived as being as result of a further mutating by OsHV-1 μ Var. In discussion it was pointed out that this apparently variable temperature threshold is likely to be due to the interplay of a variety of other factors.
- Stock movements appear to be an important factor for several reasons. It was felt that over-handling of stock stresses the animals and leaves them more vulnerable to disease.
- The testing for $OsHV-1 \mu Var$ is confusing. Different tests are used at the different laboratories and this is confusing to producers and gives the impression that perhaps one may be more sensitive than another.

Knowledge Gaps

- At the moment little or nothing is known of any possible presence or distribution of OsHV-1 μ Var in Asia. As it is still an emerging disease and not an OIE listed disease countries are not compelled to report suspected cases.
- The possible risks other carrier species play in the life cycle of the disease is not well understood and could provide some important clues.
- Translocation of spat grown out at 18[°] C is a possible mechanism for making spat more hardy and reducing mortalities. More work could be done in looking at this as an option.
- The important question of whether vertical transmission occurs.

Research priorities or actions needed

- More collaboration with Asian oyster industry is needed. Reports of mortalities, disease investigations and other possible evidence presence of OsHV-1 µVar in Asia would be most helpful in determining spread, possible transmission mechanisms and strengthening our scientific understanding of the disease.
- It was suggested that avoiding any unnecessary movement and minimising handling in routine management practices should be recommended as a strategy to reduce oyster susceptibility to disease. Restrictions on stock movements during an investigation into mortalities were also seen as crucial in the mitigation of further transmission of the disease.
- Data collection of stock movements and management activities should be improved and would potentially be a powerful tool for surveillance and epidemiological investigations.
- Improvements in selective breeding for resistance also have the potential to be useful. France has seen a reduction in mortalities in those farmers who have used lines bred for resistance.
- Genetic research seems an important priority.
- Important for all concerned with the disease to reiterate wherever appropriate that the Oyster Herpesvirus and OsHV-1 μ Var pose no threat to human health and are not zoonotic. This will allay public misconceptions about any connection with human herpesvirus simplex and is an important message to make in order to protect market confidence in the product.
- Standardisation of testing in laboratories in both state and federal laboratories should be ensured. At the moment only the laboratories at EMAI and AAHL can test for the presence of the virus. This testing should be made available at state level laboratories in order to save time and perhaps money. Outbreak investigations should send samples to both state and national (AAHL) laboratories for urgent testing.
- Development of good management practices for routine data collection, immediate response, containment, control and surveillance. These need to be clear and well documented action plans which will ensure consistency across the industry. This is urgent with summer approaching.
- A clear understanding of government bodies and personnel who are responsible for various activities in the event of an outbreak or mortality report. For example, head agency, management groups in each state and any protocols they may have, national oyster industry POMS working group etc.
- The development of a framework to action the priorities from the workshop. Establishment of a committee or advisory group. In discussion, it was suggested that the existing FRDC AAHS may be able to take this role initially.

- The importance of continuing the dialog between researchers, regulators and industry on this issue. This may be the same group or a smaller group to meet on a regular basis (every year or two) and if the virus spreads being able to meet earlier.
- Recognising the importance of preventing the spread of the virus from NSW i.e. biosecurity management, translocation, education of industry and public (disposing of oysters into land fill etc.)
- That industry is represented on any working groups that are formed. Hatcheries will be crucial in any recovery from an outbreak.
- Selective breeding be recognised as one of the most important long term strategies. With Tasmania and SA isolated from NSW it is hoped that there is time to have 5 to 10 years of breeding under our belt before the disease spreads. This may be unrealistic but we have to be positive.
- Workshop findings need to be presented to industry, WOS4 in Tasmania in September a must.
- Linking with international research groups is important.
- All Pacific oyster farming states to implement a Pacific Oyster Health Surveillance program, Tasmania has one and has data from the past 15 years on oyster health.

SCIENCE

Key points

- Causes of mortality in the oyster
- Host-pathogen interaction
- Role of other pathogens, e.g. *Vibrio*, and other mollusc species, e.g. scallops
- Genetic background of Pacific oysters
- Biosecurity measures
- Hatchery methods for producing disease free spat
- Breeding for genetic resistance
- Water temperature (16°C) and overall temperature tolerance of the virus are important factors
- Cost balance of whether to leave oysters to die *in situ* or harvest
- Environmental impacts

Research priorities

- Harmonize and validate diagnostics including a definition of mortalities
- Confirmation of the global distribution of the disease
- Pathogenicity studies

- Development of an experimental infection model
- Genetic analysis of the virus for insights into virulence and pathogenicity
- Better understanding of the genome and ORFs of significance
- Selective breeding for resistance
- Environmental risk factor analysis

REGULATORS AND MANAGEMENT

Actions recommended

- Integration and coordination of current activities and future initiatives is required
- Surveillance pre-summer 2011 in NSW, TAS and SA to improve capacity for early warning system
- Industry to work with states to ensure reporting mechanisms are in place for unexplained mortalities. Facilitating disease reporting and investigation clarify disease reporting channels including when, what and how to report. Industry training in sample collection and dispatch.
- Rapid emergency response and quick turnaround of results from laboratories
 roles, responsibilities and limitations
- Development of biosecurity guidelines good practice for farmers particularly with translocation
- Industry to be better advised of testing protocols, time frames for results and costs
- DAFF OsHV-1 µVar entry pathways project fully supported
- National survey which will detail the status of the disease in Australia should report results in August/September, 2011. It should inform national response and objective setting, future priorities and planning strategies.
- FRDC industry capacity building project (including Australian industry representatives for France and Ireland) fully supported
- Consideration of alternative species for production. Trialling *Ostrea angasi* (Australian flat oyster) as a third commercial oyster species in Australia. Trialling pipis as a potential species for aquaculture re-seeding. Possible change in producer business models to diversify stock and diminish risk.
- Examining resistant oyster lines
- National database for mortalities/events which will also inform growers and allow for benchmarking
- Testing production strategy to make sales prior to the water temperature reaching 15°C
- Examine the applicability of Performance of Veterinary Services (PVS) tool proposed by Geoff Grossel

• National listing of the disease is under consideration at the moment. Possible quarantine impacts and potential changes to other legislative powers.

Other points raised in final discussion

- AquaHealth.Net is a potentially useful information sharing tool.
- The Australian Centre of Excellence for Risk Analysis (ACERA) may be an important point of collaboration for analysis of risk factors.
- Collaboration and information sharing to be enhanced
 - o Histology via ABIN
 - o Phylogenetic (gene sequencing) studies
 - NSW and AAHL (will also undertake sequencing with approval from NSW CVO)
 - o NZ (undertaking sequencing) and AAHL
 - Publishing of gene sequencing with GenBank
 - Other oyster producing countries
- Key recommendation: Use of the existing FRDC AAHS as an advisory and coordinating body for future activities and research. Needs to rejuvenate industry cooperation and ensure state jurisdictional contributions. International collaboration also required to widen its international engagement, i.e. informal links with IFREMER, OIE Reference Laboratory.
- Greater cooperation and also a more sensitive appreciation of different standpoints between oyster industry, shipping authorities, quarantine, science etc to enable further work on route of introduction: shipping, biofouling, ballast water, equipment imports, public waste disposal etc. in order to reduce the introduction of marine pests and diseases.
- Multifactorial approach needed when examining causal agents or risk factors. There is a complex web of causation, for example temperature is an important but not definitive switch for the disease. The difference in effects of temperature on virus and on host appears complex also.
- State laboratories should request standard positive controls from AAHL to ensure the consistency of tests (demand for standard positive controls need to be industry driven) TAS has agreed to make this request.
- Cost sharing in Tasmania between Government and industry for ongoing surveillance and testing.
- Virus characterisation. Research required into phylogenetic relationships of all isolates globally.
- Reporting is hampered by the lack of a definition of increased mortality. A clear case definition for "increased mortality" including the life stage or production system affected and a mortality threshold is still not available.

ANNEXE 5: SELECTED INTERNET LINKS

EURL for Mollusc Diseases, IFREMER. Tutorial on OsHV-1. http://wwz.ifremer.fr/crlmollusc/Main-activities/Tutorials/Herpes-virus-OsHV-1

IFREMER OsHV-1 detection and quantification by real-time polymerase chain reaction.

http://wwz.ifremer.fr/crlmollusc/content/download/42545/578238/file/OsHV-1%20RTPCR_1.pdf

Oyster mortalities in connection with OsHV-1. Commission Regulation (EU) No 175/2010 implementing Council Directive 2006/88/EC as regards measures to control increased mortality in oysters of the species *Crassostrea gigas* in connection with the detection of Ostreid herpesvirus 1 µvar (OsHV-1 µvar). http://www.megapesca.com/megashop/FH201103_i89/Oyster_Mortalities.htm

Guidance document on the establishment of surveillance programmes for ostreid herpesvirus 1 µvar (OsHV-1 µvar). European Commission, March, 2011 <u>http://ec.europa.eu/food/animal/liveanimals/aquaculture/guidance_document_</u> <u>OsHV-1%20surveillance_en.pdf</u>

Dataquest: Inventory of data sources relevant for the identification of emerging diseases in the European aquaculture population. EFSA. http://www.efsa.europa.eu/en/supporting/pub/90e.htm

Scientific Opinion on the increased mortality events in Pacific oysters, *Crassostrea gigas* 1. EFSA Panel on Animal Health and welfare (AHAW) 2, 3. European Food Safety Authority (EFSA), Parma, Italy.

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Appendix 4:

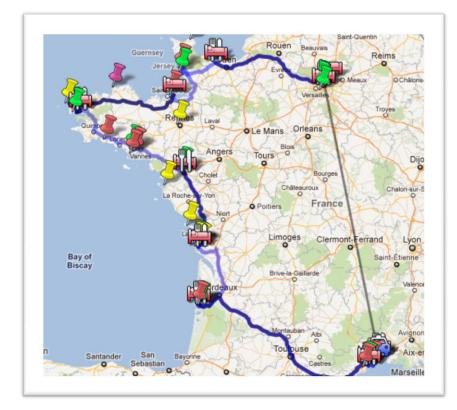
French Study Tour Report

Understanding and planning for the potential impacts of OsHV1 µvar on the Australian Pacific oyster industry (FRDC 2011/043)

French Study Tour Report

8 December 2011

Bruce Zippel, Rob Moxham, James Calvert, Angus Cameron and Tom Lewis





Australian Government Fisheries Research and Development Corporation



Acknowledgements

This report and the participation of Bruce Zippel, Rob Moxham, Angus Cameron and Tom Lewis on the study tour in France were funded by the Fisheries Research and Development Corporation (FRDC) as part of project number 2011/043 (*Aquatic Animal Health Subprogram: understanding and planning for the potential impacts of OsHV1 on the Australian Pacific oyster industry*).

James Calvert's contribution and participation on the study tour was fully funded by Tas Prime Oysters.

The FRDC is Australia's leading agency concerned with planning, investing in, and managing fisheries research, development and extension.

The FRDC is a statutory corporation founded in 1991 under the Primary Industries and Energy Research and Development (PIERD) Act 1989. It is responsible to the Minister for Agriculture Fisheries and Forestry.

The FRDC's mission is to maximise economic, environmental and social benefits for its stakeholders through effective investment and partnership in research, development and extension.

The project is being managed by Tom Lewis at RDS Partners. RDS Partners is a multi-disciplinary team dedicated to facilitating positive social, economic and environmental outcomes in rural and regional Australia. RDS specialises in projects within the agriculture, seafood and not-for profit sectors.

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Introduction

Following the late 2010 confirmation of Pacific oyster mortalities in New Zealand and NSW associated with the presence of the OsHV1 μ var virus, the Australian industry and FRDC supported¹ an industry study tour to France to gain first hand information of the effect on the French industry of the virus and the response to this threat by industry, researchers and regulators.

The study tour team comprised:

- Growers: Bruce Zippel, Rob Moxham, James Calvert;
- Epidemiological expertise, cultural attaché, translation and tour logistics: Angus Cameron, Cate Mackenzie (AusVet Animal Health Services, www.ausvet.com.au); and,
- Project manager: Tom Lewis.

Between 1 and 10 November 2011, the study team travelled from Paris to Normandy, around the French coast to the Mediterranean and back to Paris, meeting with growers, processors, industry representatives, researchers and government agencies.

A daily "blog" for this trip (www.oystertour.wordpress.com) was maintained to provide information in real time to interested parties and to enable them to provide feedback and ask questions during the tour.

The blog remains online as a resource to add detail to the contents of this report, which, in turn, provides a summary of the team's thinking at the end of the tour.

¹ James Calvert's participation was funded by Tas Prime Oysters. All others were supported through a combination of FRDC, Tas, SA and NSW oyster industry research council contributions.

Key recommendations

Research priorities

- 1. Increase selective breeding focus on developing virus resistant family lines that maintain the economic value already realised.
- 2. Establish a trial in the Georges River NSW to test the effect of growing height and oyster density on mortalities (possibly 3 heights, 3 densities, 3 replicates = 27 baskets).
- 3. Establish a series of trials in the Georges River (NSW) to test the effectiveness of other growing systems including adjustable longline systems and the floating basket system in use in the NSW oyster industry.
- 4. Adapt the French infectivity models as published by IFREMER in an Australian biosecure facility as the basis for direct research into different aspects of the virus.
- 5. Standardised protocol for PCR testing for the virus within Australia to provide confidence in result comparison between testing agencies.
- Run a temperature "stress" trial to establish if increasing the culture temperature by about 1C per day to above 17C will elicit disease in sub-clinically infected oysters (if successful, this would be used as a fast and cheap test for the presence of virus in oysters).
- 7. Research the ability of other bivalve species to act as translocation and/or disease vectors.
- 8. Determine whether vertical transmission of the virus occurs.
- 9. Establish if virus has spread (e.g. north and south of Sydney Harbour).

Industry actions

- 1. Develop and implement plan for discussing tour findings with industry, researchers and regulators in SA, NSW and TAS.
- 2. Undertake an immediate risk assessment on likely vectors of transferring the virus within a state and between states.
- 3. Investigate the use of sentinel populations in high risk areas of potential viral infection. This may involve a mixture of cultivated and feral oyster populations.
- 4. Develop national capacity and capability to report and monitor non-harvest stock movements between states and within each state.
- 5. Develop a plan (or plans) for preparing an OsHv1 μvar-focussed, industry -owned and coordinated emergency response plan in each of SA, NSW and TAS. This plan (or plans) should include details of agreed:
 - a. technical response options, including contingency planning and learning how to live with the disease and knowing what the options are for maintaining commercial production in an infected area

- b. regulatory response options
- c. financial options available at the individual farm level and at an industry level, including sources of assistance during the recovery phase
- d. social and other sources of support for producers coping with this sort of problem

Summarised findings

This study tour provided information and contacts that should prove useful to the Australian industry. The study team did find, however, a certain lack of consistency in opinion about the effect that different factors have on virus-related mortalities. This could mean that:

- The effects are different in different locations / environments;
- There are other important factors influencing survival that we are not yet aware of;
- The factors being considered have little impact on survival, and observed differences are just random variation; and/or
- Desperate farmers are clutching at straws, hoping that different ideas may work, but with little support or proof (we have heard of a range of different ideas that sound rather improbable but which some farmers are keen to implement without any firm evidence that they may actually help).

Despite this there are some issues about which information has been more consistent and that the team feels merit careful consideration.

Hatchery/Nursery related issues

Virus transmission

We heard differing reports as to whether true vertical transmission (this means transfer of the virus from broodstock to larvae via infected eggs and/or sperm) occurs with this virus. The consensus is that it doesn't, and that if larvae get infected, it is from virus particles in the water, probably shed by the broodstock. If this is true, then good technique should be able to produce virus-free larvae from infected broodstock, although this may need some level of further research.

Hatchery Management

There was some level of conflicting reports as to whether hatchery management techniques had some effect on reducing virus-related mortalities. It did seem to be important from what we could gather to use broodstock with previous exposure to the virus, but weren't showing any clinical signs of being affected by it.

There was a general consensus that wild caught oysters fared better against the virus than did hatchery spat. It was also acknowledged that the quality of the spat from a hatchery definitely influenced mortality rates, and some hatcheries had a better reputation for having stock that was able to withstand the virus than other hatcheries.

We were also told that occasional batches of oysters that went through hatcheries survived quite well, but the reasons for this better survival were not known. We asked about the importance of not pushing oysters quickly through the hatcheries, and we received a mixed response. One response was that it was important not to push the growth of hatchery and nursery stock, yet others said it didn't matter and that the quality of the broodstock was more important.

The feeling of our group was that perhaps it did matter to some extent, as the Australian experience suggests that the quickest growing oysters in a batch tended to more susceptible to husbandry

problems (remembering that the fastest growers of a batch are usually culled by the hatchery in Australia).

In regards to the IFREMER hatcheries, we were told that some interesting research was going to be released in regards to hatchery management for the virus, but it was yet to be peer reviewed etc. Hopefully this will information will be available be before too long.

Growth rate

As discussed later (see Husbandry related issues) it seems that faster growing oysters, including larvae and spat, are more susceptible to mortalities. It could be important for hatcheries to manage larvae and spat growth rates to help manage this. Managing growth rates could also be an important component of on farm husbandry to manage the virus.

Timing

Larvae and spat can be produced to match industry understanding of the safest timing (e.g. size, age, water temperature).

Selection of spat

The French did not provide any evidence that there was any difference in mortality rates between diploid, triploid, wild-caught or hatchery-produced, although the French peak industry representative body was of the opinion that wild caught spat fared better than normal hatchery spat. The team feels it is unlikely that these variables alone have any significant influence on mortality. It is more likely that the genetics and husbandry of spat will play a larger role in determining susceptibility to the virus.

Husbandry related issues

Good husbandry practice is thought to be an effective means for limiting virus-related mortalities. This adds another strong incentive to keep your farm and stock in good order.

There is a limited range of husbandry techniques that can be considered in attempting to minimise the impacts of the virus.

Timing of stock input and movements

We heard regularly that the age and/or size of oysters, including spat, can have an influence on their mortality during times of increased viral activity. We also heard, and the French acknowledged, conflicting reports on this issue for oysters grown in different areas.

Once better understood in the Australian (or state, or bay, or culture system) context, this knowledge could provide useful insights to any management tools (e.g. spat can be transferred to farms at different times, so that they are bigger or smaller, older or younger, at times of peak risk (when water temperature is higher).

Growth rates

We heard regularly that rapidly growing oysters are more susceptible to viral-related mortality than slower growing ones. The exact mechanism for this has not been described with any consistency. It may be simply because rapidly dividing cells provide a better opportunity for the virus to replicate

itself. Others point to physiological stress in rapidly growing oysters, and others suggest that shell strength plays a role (that is, it was felt that oysters grown in conditions that slightly inhibited growth and produced strong "solid" shells were more resilient to disease).

A number of strategies appear to have developed in response to the hypothesis of the importance of growth rate, involving speeding up or slowing down growth of different classes of oysters at strategic times.

Acting to decrease oyster growth rate, especially during times when the virus is active (e.g. when the water temperature is over 17°C), is thought to lead to decreased mortalities.

This has been achieved by growing oysters:

- at different heights in intertidal systems;
- closer to the surface in sub-tidal systems; and/or,
- at higher densities [and in this case, density should be considered at two SCALES: 1) at a local scale (number of oysters per bag/tray etc), and 2) at a larger scale (number of bags/trays per hectare in a growing area).

Transfers

It appears that stock transfer is one of the most effective ways of moving the virus from an infected area to a non-infected area. This means that the issue of when and how to limit stock transfers needs to be considered very carefully.

However, the French experience is that stock can be moved between different areas to influence growth rates or to avoid or delay exposure to heavy virus loads and warmer water temperatures.

Grading and handling

It appears that handling, even just washing, oysters during times of increased virus activity can lead to increased mortality. One grower on the Atlantic Coast told us that the virus seems to come in waves about a month apart during summer. This grower said he prepared his oysters for the disease by having them more "rumbled" (or other means of slowing the growth) about a month prior to the expected onset of the virus. Then, after the first monthly wave, he would handle oysters only if he had to, and would do everything to minimise shellfish stress, including immediate placement back into the ocean.

The timing for purchasing spat or ongown oysters seemed to influence survival depending on the strategy in place by the grower. Some will buy in the spring and then allow for moderate growth prior to preparing the oysters for summer. Others will buy in the autumn and then try and get greater growth to preparing the oysters for increased survival during the following summer. There did not seem to be a clear pattern suggesting which approach would be better.

Management of survivors

Oysters that have been previously exposed to the virus but have not died were seen to be treated in different ways by different growers:

- some consider that these oysters have been weakened by the virus and are more susceptible to dying with any later challenge;
- others think that they represent a population with some immunity; and,
- yet others think that they are likely to be carriers and therefore risk spreading the disease.

Temperature

One of the few things that people are pretty confident of is the effect of temperature on triggering the mortalities. If the virus is present, rising temperature triggers the onset of disease. The threshold at which mortalities start to appear varies a bit, which is what one would expect if a range of other factors are also involved (e.g. other sources of stress, other opportunistic pathogens such as *Vibrio* spp, age, resistance etc).

Most people we spoke to suggested that the threshold in most of France is around 17°C. IFREMER has also suggested that there is an upper temperature limit to the mortalities, around 24° C, but no similar comments have been made by producers and it is not certain what the practical importance of this would be in France. It may be relevant in Australia, however.

In some parts of France, farmers with multiple leases in different areas take advantage of this temperature effect. Some small areas have lower temperatures than surrounding areas and oysters may be transferred here to avoid the wave of mortalities that accompanies increasing water temperature. In Australia, there is a significant range of water temperatures between different oyster growing areas, but restrictions on interstate movements mean that the opportunities to exploit this type of approach will be limited to certain intra-state transfers.

Age/size

The early observations in France have been that young oysters are more susceptible. This still appears to be the case in most areas, to the point that farmers on the Atlantic coast seem to feel that adults are not at significant risk. They were surprised by the observations from Australia and New Zealand that older oysters also suffered very high mortality rates.

In contrast, in the Mediterranean (and possibly some other areas), adults appear to suffer heavy losses. Based on the hypothesis that genetic resistance is the main determinant of survival, some farmers prefer to have their spat heavily challenged and suffer high levels of mortality, so that the survivors have a lower chance of dying as adults. Losing adults after all the effort of growing them clearly has a very demoralising effect.

What is not clear is the interaction between age, size and growth rate. Definitions of adult and juvenile are generally based on size, but may describe oysters of very different ages, depending on the growing area. Significant differences in nutrient levels and growth rates, and therefore age/size relationships, are likely to be evident in Australia as well.

The group was informed that when mortalities started due to the virus, it was more likely to affect oysters across all sizes and age classes. In the Thau Lagoon, we were told that if oysters were brought in that hadn't been affected or exposed to the virus (some virus-free pockets exist in the Mediterranean apparently), those oysters die very quickly, regardless of size or age.

Viral load

Another relatively consistent message was the importance of viral load. It appears that oysters exposed to the virus may well continue to carry the virus, even if it is not detectable with PCR tests. It is not known if they excrete the virus in this subclinical state.

One suggestion (that has apparently been patented in the Mediterranean) is to grow mussels between the rows of oysters, based on the theory that they may act as a barrier to the movement of virus, or 'soak up' virus from the environment without being affected. There is little information available about the effectiveness of this approach. One IFREMER study showed that the mortality of oysters grown in a range of different environments (surrounded by mussels, other adults, or spat) were very similar, suggesting that it may not be effective.

On the other hand, the importance of viral load in the environment was demonstrated by an experiment in which spat that tested negative on PCR were grown in a previously unused area that was separated from existing (heavily contaminated) oyster growing areas by land and hydrological barriers, and suffered no mortalities despite having suitable water temperatures. This indicates that it is possible to avoid the disease by using a clean environment and spat that are either free or have infection which is undetectable by PCR.

Genetics

Genetics / Breeding for resistance is the area that is seen as the principle hope for the French oyster industry to combat this virus. There has been much effort spent so far in this area, and under Research Related issues (below), a selective breeding project is outlined that will require significant cash and in-kind resources.

The past lines that were originally bred for resistance for the classical OHSV-1 were released after being crossed with IFREMER tetraploids. It would appear that there were major problems getting enough of these oysters produced, and they failed under commercial conditions.

We were told that the next, genetically improved line of this breeding trial has performed well in trial conditions within the claires², and hopefully that line will be produced in commercial quantities for the upcoming summer (although it wasn't clear if there were any in hatcheries at the time of our visit).

Research related issues

Virus-related research in France was discussed by the group with IFREMER representatives at some length, with the following areas covered:

• Breeding for resistance

Development of virus resistant family lines is being undertaken by both government and private hatchery operations.

² The "claires" are ponds dug in the clay and they are generally ancient salt pans reconverted in the19th century, after the decline of the production of salt. After their growth in the sea, oysters are placed in the "claires" where they acquire their tint and their taste (e.g. ref: http://bernezac.com/huitres_uk.htm).

The French have an ambitious aim of producing up to 2-3000 family lines over the next few years, with the aim of producing at least 100 virus-resistant lines as a basis for introducing a genetically diverse resistant population into the wild population (on which 80% of spat production relies). This project will be undertaken by IFREMER, but is likely to need to cooperation of private hatcheries to reach such a high number of family lines in the first season.

It is important to note that, in France, commercially bred lines can only be released as triploids. The French are very determined to protect the genetic diversity of their wild pacific oysters. Tetraploids of any sort, and in this case used for crossing with diploids to create triploids, can only be held by IFREMER.

IFREMER only release male tetraploids to commercial hatcheries and the shells of those oysters are micro chipped and must be returned to IFREMER after commercial use.

• Understanding potential vectors, including other species

We heard many and varied reports of other species that may carry and/or be affected by this virus. Needless to say this area needs much more work.

IFREMER is planning studies in their coming spring and summer to look into possible live and particulate vectors for this virus. It will be important for us to keep an eye on this research.

• Understanding the genetic relationship between the virus in different countries

As more work is done on the virus, it is becoming apparent that there is potentially greater genetic diversity between strains from different countries than previously thought.

Increased efforts on understanding the differences, and similarities, of different strains will provide greater understanding of its origin and path of spread around the world.

• Monitoring the virus

It is believed possible, even likely, that the virus may be difficult to detect, even with PCR analysis, when it isn't active (particularly when from water below temperatures that coincide with mortalities). This was carefully noted by the group, hence the discussions about the usefulness of laboratory-based "stress tests" in which oysters are held in tanks and warmed to >17°C (by about 1°C per day) to stimulate the onset of the disease if the virus is present.

One other interesting point raised was the belief of one scientist that the virus does not spread easily in the water column, and may not spread from infected oysters to uninfected oysters over distances greater than about 1.5 kilometres.

Another area discussed within this topic was the possible use of pooling samples rather than testing individual oysters. It was felt by the IFREMER representatives that it was definitely possible to pool a sample of 10 - 12 oysters from a region and detect the virus if it is present and active. If the intent was simply to see whether the virus was present, then this process

would be acceptable. If the virus is detected in a pooled sample, then individual oysters could then be tested to determine the location and level of infection.

• Infectivity Models.

The group asked about infectivity models for laboratory transmission of the virus for the purposes of experimentation. It would appear that 2 models have been published, one based on direct injection of the virus into the adductor muscle, and one based on cohabitation with infected oysters.

It was felt that if there was any move for researchers in Australia to develop another model, then there would have to be a very clear reason to do so. In Australia, this would obviously have to be done in a biosecure facility.

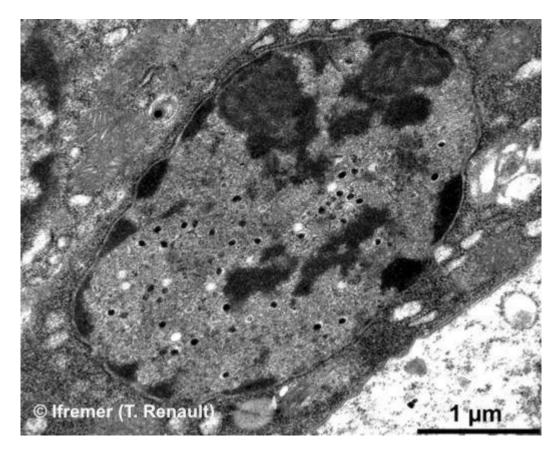
The role of *Vibrio* spp bacteria in oyster mortality.

One IFREMER scientist was quite adamant that pathogenic *Vibrio* spp. bacteria had a key role to play in mortalities associated with the virus. He spoke of research that showed 1) a greater correlation between mortality and the presence of *Vibrio splendius* than with the presence of the virus, and 2) that when pathogenic *Vibrio* strains were removed in a laboratory experiment, the oysters did not die.

The scientist also reported that the mortalities in the experiment only occurred in the temperature range of 17 - 24 °C.

Based on our many conversations in France, the team considers that the following research topics should be included in Australian industry plans:

- Management options for decreasing mortality
- Cheaper, more practical tests for surveillance
- Development of effective clinical surveillance and transfer data capture systems
- Spatial network analysis
- Role of other species in spreading the disease
- Development of an experimental model
- Genetic analysis



OsHV-1 virus particles (black dots) in Pacific oyster tissue (Transmission Electron Micrograph courtesy Dr T. Renault, IFREMER)

Appendix 5:

Briefing Note:

Emergency Aquatic Animal Disease Preparedness in Tasmania in response to detection of OsHV-1 μVar* in New Zealand

Emergency aquatic animal disease preparedness in Tasmania in response to detection of OsHV-1 in New Zealand

Briefing note for industry prepared by the DPIPWE Biosecurity and Product Integrity Division 14 December 2010



Background

On December 8 the New Zealand Ministry of Agriculture and Forestry (MAF) reported that their Pacific oyster industry had experienced a significant increase in deaths amongst young Pacific oysters on upper North Island marine farms during November and early December of this year.

MAF has advised that although it believes that the mortality is likely to be caused by a range of factors triggered by unusually warm water temperatures; it has also identified the presence of ostreid herpesvirus (OsHV-1) in samples from affected farms using molecular tests and DNA sequencing. OsHV-1 is the virus that has significantly affected oyster production in France during recent years.

Herpes-like viruses have previously been identified in oysters at various locations throughout Australia, including Tasmania. All of these detections in Australia occurred before molecular tests were available to identify whether the herpes-like virus visualised in samples were actually OsHV-1 or another virus. The Australian herpes-like viruses have never been associated with significant mortality and have never been detected in Pacific oysters. In the Tasmanian case, a herpes-like virus was detected in a single flat oyster sampled as part of surveillance activities during 1993.

The Tasmanian Department of Primary Industries has recently been communicating with Biosecurity Australia (the lead national agency for biosecurity policy) on this issue and the potential threat that it may hold for Tasmanian producers. Contrary to claims recently made by New Zealand authorities, it is the opinion of Biosecurity Australia that OSHV-1 does not occur in Australia.

Biosecurity Australia is currently reviewing risk pathways associated with the importation of New Zealand oysters and aquaculture equipment into Australia, with the view of tightening quarantine regulations. New Zealand currently exports approximately 200 tonnes of half shell product into Australia annually, of which little or none comes into Tasmania.

Emergency Animal Disease Response

The exact form of any response to an outbreak of OsHV-1 in Tasmania would depend on factors relating to the particular situation. The Tasmanian Chief Veterinary Officer (CVO) would immediately establish measures to contain any outbreak before developing a response plan in consultation with the peak industry body and government.

If the disease were to become established in wild Pacific oysters it is considered that it would be extremely difficult to eradicate from infected open water sites, however eradication of disease from hatcheries is considered possible. Therefore any emergency response measures applied would have to take into consideration the individual situation and may need to be revised as information becomes available.

If an emergency response were mounted, it would be based on the generic emergency response process the department uses as the basis for other emergency animal disease operations.

In any such process

- Any infected "premises" (which may include aquatic leases areas, bays or hatchery facilities) would have movement restrictions for stock, equipment and personnel placed imposed upon it. Any other premises considered by the Chief Veterinary Officer (CVO) to be at significant risk of infection may also have similar conditions placed on them until the full extent of the outbreak is determined. The CVO has various powers under the *Animal Health Act* to take such action quickly.
- A disease control centre would be established to coordinate emergency response activities. During the early stages, its main tasks would relate to collecting samples to confirm the diagnosis and establish the extent of disease spread through tracing of stock and product movements together with active surveillance activities.
- There may be a broad restriction, or even ban, on the movement of oysters. That may apply across the whole of Tasmania or just in a designated part of the State, depending on the circumstances. Any such restrictions would be reviewed frequently as more information became available. For example, it is likely that there would be a ban of all oyster movements around Tasmania for a few days until tracing and surveillance had established the scale of the outbreak.
- There would be an information campaign that would focus on advice to industry
 members about measure needed to minimise the risk of disease spread and to
 consumers about the safety of eating oysters. The communications campaign would
 also establish a means of providing regular updates on the response to industry and
 anyone else who wishes to be kept informed. During the abalone disease response
 in 2008, DPIPWE established an email update service that provided daily situation
 reports on the response. Around 250 people simply self-registered to get those.
- Given the very high mortality rates typical for OsHV-1, a high priority would be to establish controls for the disposal of dead oysters and any by-products or waste and advise producers on appropriate biosecurity and sanitary measures..
- As surveillance and tracing provided more information relating to the extent of the disease, DPIPWE may establish "restricted areas". This would enable gradual easing of movement restrictions in non-infected areas. Such restricted areas would not necessarily inhibit production within infected areas, merely limit where oyster stock may be sent in order to reduce any risk of spread. Such measures are currently in-place for the Tasmanian salmon industry without unduly affecting production.
- The oyster industry would be invited to provide an industry liaison officer to work within the disease control centre. His/her primary role would be to ensure effective liaison both ways between the control centre and the industry. Also, the CVO would stay in regular contact with the industry peak body during the response.
- All of the above would occur quickly in order to contain the outbreak and to minimise unwarranted consumer reaction to oyster product. As the tracing and surveillance helped clarify the outbreak situation, the disease control centre would make adjustments to the response to ensure it was correctly targeted and to help facilitate a return to normal business as soon as possible.

Financial arrangements

There is no cost sharing agreement currently in place within Australia for any aquatic animal diseases and as a result no compensation available for any direct or contingent losses to individual producers and allied businesses arising from either the outbreak or the response. Similarly, there would be no cost sharing available between the Tasmanian State and Federal governments to cover the cost of response measures, as occurs in terrestrial livestock industries. In contrast, most terrestrial livestock industries are now, through their national peak bodies, involved in a cost sharing agreement and are committed to an agreed biosecurity plan. The latest agreement, involving the horse industry, is close to being signed off. An emergency animal disease response agreement has two significant benefits. Firstly, the biosecurity plan minimises the risk of emergency disease. Secondly, the mechanics of the response, and the issue of cost sharing, are all agreed beforehand, which enables both industry and government to focus all their available resources on the response at the outset of an outbreak without undue concern over whether funding is available to mount an effective response.

Preparedness

Tasmania has a large pool of people trained to work in an emergency animal disease response – DPIPWE currently has around 120 people on standby. If a response to an outbreak of OsHV-1 were similar in scale to the response we mounted to the abalone disease outbreak in 2008, the disease control centre would involve around 25 of our pool of trained people together with a further 15 field personnel.

It would be most useful if the Tasmanian oyster industry had a few people trained as industry liaison officers. It would also be useful if the Tasmanian industry, with the assistance of DPIPWE, developed a basic emergency animal disease response plan. Such a plan would identify, in advance of any outbreak, who would have the authority to negotiate with government over the response, who would be the industry spokesperson and what means would be used to get vital information out to members quickly in the event of an outbreak.

Tasmania has a well-developed emergency response plan that complements the various AQUAVETPLAN emergency aquatic animal disease response plans agreed nationally. Emergency animal disease plans were activated in Tasmania for both equine influenza in 2007 and abalone herpes virus in 2008.

Laboratory support for diagnostic testing required during any OSHV-1 emergency response would be provided by the DPIPWE Animal Health Laboratories and the CSIRO Australian Animal Health Laboratories in Geelong.

Any enquiries should be directed to:

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